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EVALUATION OF TRITIUM CHOLESTEROL AS A TRACER IN MAN*

HAROLD WERBIN, PH.D., DELBERT M. BERGENSTAL, M.D., R. GORDON GOULD, PH.D. AND GEORGE V. LEROY, M.D.

Departments of Biochemistry and Medicine, and the Argonne Cancer Research Hospital, The University of Chicago, Chicago, Illinois, and the Los Alamos Scientific Laboratory of the University of California, Los Alamos, New Mexico

INTRODUCTION

STUDIES have been in progress during the past three years in this laboratory[1] to determine the percentage of the urinary steroids in man that is derived from cholesterol. A part of this project has involved the feeding of tritium-cholesterol (cholesterol-t) to human beings for extended periods of time, *e.g.*, as long as two weeks, and the isolation of labelled plasma free cholesterol and urinary steroids. Before these studies were undertaken, it was necessary to establish 1) that the tritium in cholesterol-t, prepared by catalytic exchange, was not labile in the course of the catabolism of the sterol in the body to bile acids and steroids; and 2) that tritium derived from the degradation of the side chain did not re-enter newly synthesized cholesterol or steroid hormones to any appreciable extent. Therefore an investigation was initiated to evaluate the use of cholesterol-t as a tracer in man. This paper presents the data that were obtained from this study.

The distribution of tritium, between the side chain and the tetracyclic

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[1] Argonne Cancer Research Hospital, The University of Chicago, Chicago 37, Illinois.

rings, was established by chemical degradative procedures. A male subject was given intravenously a mixture of carbon-14 and tritium cholesterols of known specific activities. Plasma free cholesterol and five urinary steroids were isolated, and the ratio of tritium to carbon-14 specific activities was determined by means of a liquid scintillation counter. The findings demonstrated that exogenous cholesterol was a precursor of three urinary adrenal steroid metabolites namely, pregnan-3-α-17-α-21-triol-11,20-dione (THE), pregnan-3-α-11-β-17-α-21-tetrol-20-one (THF), and etiocholan-3-α-ol-11, 17-dione (11-ketoetiocholanolone) *in vivo* (1, 2). The data also showed that cholesterol-t could be used as a tracer in man for catabolic studies involving that sterol. The advantages of using tritium in place of carbon-14 as a tracer for investigations in human beings have been described by Biggs and Kritchevsky (3).

MATERIALS AND METHODS

All melting points were taken on a Fisher-Johns apparatus.

Tracers: Cholesterol-t was prepared by an exchange reaction with tritium water (THO) (4, 5). Labile tritium was removed by refluxing the cholesterol for two hours with 5 per cent potassium hydroxide in methanol (6). After 2 crystallizations from 80 per cent alcohol, the cholesterol-t had a melting point of 146–147°, and a specific activity (s.a.) of 7.3 μc. per mg. The 4-C^{14}-cholesterol (m.p. 147–148°, s.a. 5.0 μc. per mg.) was obtained from Radioactive Products Inc., Detroit, Michigan.

Patient and administration of cholesterol: The patient was a 60-year-old white man with chronic rheumatoid arthritis. Cholesterol-t (33.8 μc.) and 4-C^{14}-cholesterol (4.24 μc.) were dissolved in 2.3 Gm. of propylene glycol. The solution was dispersed in 30 ml. of sterile human plasma that was administered intravenously with no untoward effects in a single dose.

Distribution of tritium in cholesterol: Cholesterol-t was diluted with "cold" cholesterol and converted to cholesteryl chloride-t by the method of Blecher and Gurin (7). The steroid melted at 97° after purification by chromatography on aluminum oxide (8) and crystallization from acetone-alcohol. A 3-Gm. portion was pyrolyzed by the procedure of Bloch and Rittenberg (8). After pyrolysis, the mixture of iso-octane and iso-octene was distilled off. The residue was chromatographed on aluminum oxide, and the $C_{19}H_{30}$ hydrocarbon was eluted from the column and counted.

$C_{19}H_{30}$—calculated, C 88.4, H 11.6; found, C 88.4, H 11.3

One gram of cholesterol-t was subjected to the Oppenauer oxidation as described by Jones *et al.* (9). Following chromatography and crystallization from acetone, the Δ^4-cholestenone-t melted at 87.5–88°. The steroid was refluxed with aqueous methanolic potassium hydroxide under nitrogen for two hours, and after re-isolation was found to have the same melting point.

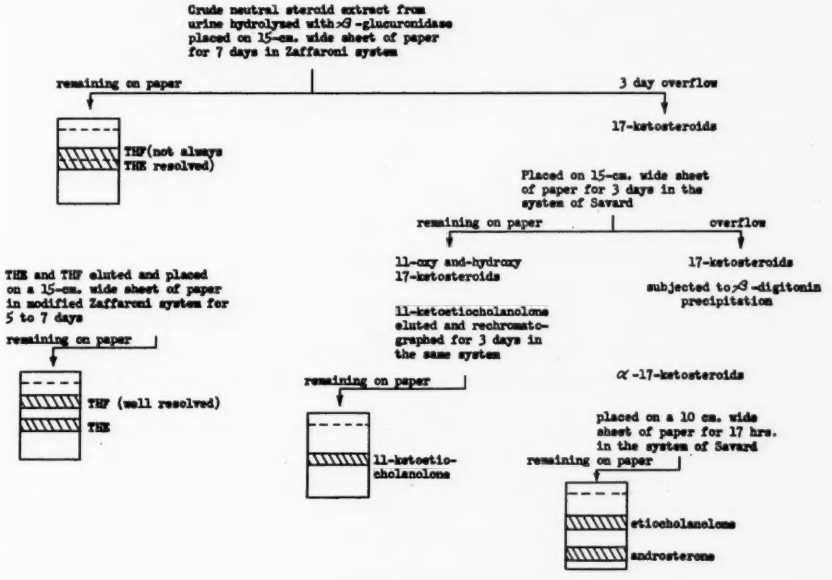
A 12-Gm. portion of cholesterol-t was converted to methyl-3 β-acetoxy-Δ^5-cholenate-t by the procedure of Fukushima and Gallagher (4). After repeated crystallization from acetone-methanol, the steroid melted at 157.2–158.6° (156.5–157.5° reported).

Isolation of plasma free cholesterol: The method for the isolation and radioassay of plasma cholesterol has been described (10).

Hydrolysis and extraction of the urine: Twenty-four-hour urine samples were adjusted to pH 6.5 and 75 units/ml. of β-glucuronidase (Sigma) was added. Following incubation for forty-eight hours at 37° and the addition of 90 Gm./L. of anhydrous sodium sulphate, the urine was extracted continuously with ether for two days, and a crude neutral steroid extract was prepared according to the method of Baggett *et al.* (11).

Isolation and identification of urinary steroids: The paper chromatographic methods of Burton *et al.* (12) and Savard (13) were used to separate the steroids. Good separations of THE from THF were obtained when the Whatman #1 paper was impregnated with a solution containing 2 parts of methanol to 1 part of propylene glycol prior to application of the crude steroid mixture. Figure 1 is a flow sheet for the separation of the

FIG. 1. FLOW SHEET FOR THE SEPARATION OF THE URINARY STEROIDS.


Crude neutral steroid extract from urine hydrolysed with β-glucuronidase placed on 15-cm. wide sheet of paper for 7 days in Zaffaroni system
remaining on paper
3 day overflow
17-ketosteroids
THF(not always THE resolved)
Placed on 15-cm. wide sheet of paper for 3 days in the system of Savard
remaining on paper
overflow
11-oxy and-hydroxy 17-ketosteroids
17-ketosteroids
subjected to β-digitonin precipitation
THE and THF eluted and placed on a 15-cm. wide sheet of paper in modified Zaffaroni system for 5 to 7 days
11-ketoetiocholanolone eluted and rechromatographed for 3 days in the same system
remaining on paper
THF (well resolved)
THE
remaining on paper
α-17-ketosteroids
placed on a 10 cm. wide sheet of paper for 17 hrs. in the system of Savard
11-ketoetio-cholanolone
remaining on paper
etiocholanolone
androsterone

steroids. THE and THF were assayed by the method of Chen *et al.* (14) and 17-ketosteroids were determined by the procedure described by Callow *et al.* (15), using stabilized alcoholic potassium hydroxide (16).

The C_{19} steroids were identified by paper chromatography in two solvent systems (ligroin-propylene glycol and ligroin-phenylcellosolve). An aliquot of each eluted steroid was converted to the acetate and was identified by paper chromatography (ligroin-formamide). Initial identification was assumed if the unknown, the authentic steroid, and the mixture had the same R_f values in all the paper chromatographic systems that were studied. At this point, carrier steroid was added and after several crystallizations, if the specific activity remained constant, identification was considered complete—provided the melting point of the steroid agreed with that recorded in the literature. In the case of etiocholan-3-α-ol-17-one (etiocholanolone), the infrared spectrum of a sample containing 36 per cent urinary steroid was identical with the spectrum of au-

thentic etiocholan-3-α-ol-17-one. 11-Ketoetiocholanolone was isolated and counted as the acetate.

The procedure for the isolation and identification of THE has been described (1). Part of a pooled sample of THF was converted to the diacetate and was crystallized from methanol without the addition of carrier steroid. The identification of THF was carried out in the same manner as that described for THE (1). The ultraviolet spectrum of the THF diacetate after two hours in concentrated sulphuric acid was identical with that of the spectrum of pure THF diacetate. Carrier was added to the remainder of the pooled THF diacetate, and the mixture was crystallized from methanol-ligroin (1:1). The infrared spectrum of the crystalline material (70 per cent urinary steroid, 30 per cent carrier) coincided with the spectrum of authentic THF diacetate.

Radioassay: The steroid was dissolved in 50 ml. of toluene containing 100 mg. of 2,5-diphenyloxazole in a tall form-weighing bottle (17). The solution was cooled to −20° and counted in a Packard liquid scintillation counter, purchased from the Packard Instrument Co., La Grange, Ill. The carbon-14 was assayed by screening out the tritium pulses by a proper choice of discriminator settings. Then after resetting the discriminators, both the carbon-14 and tritium counts were registered simultaneously. The following equation was employed to calculate the carbon-14 and tritium disintegrations per minute:

$$\text{DPM } C^{14} = \frac{\text{cpm } C^{14}\text{ (B)}}{C^{14}\text{ efficiency (B)}}$$

$$\text{DPM T} = \frac{\text{cpm (T} + C^{14}\text{) (A)} - \text{DPM } C^{14} \times \text{efficiency } C^{14}\text{ (A)}}{\text{T efficiency (A)}}.$$

A and B were two discriminator settings. At setting B the tritium counts were screened out effectively. Table 1 shows the accuracy attained by this method of counting at the levels of carbon-14 and tritium that were found in the isolated steroids. The time of counting was sufficiently long so that the probable error of counting was no greater than ±5 per cent.

After counting, the steroid was reisolated chromatographically on aluminum oxide or silica gel. The scintillator was eluted with benzene, and the steroid was eluted by increasing the polarity of the benzene with alcohol.

Five milligrams of THE diacetate had no quenching effect on the carbon or tritium that was counted. It was assumed that the other steroids behaved similarly.

RESULTS AND DISCUSSION

Figure 2 shows the distribution of tritium in cholesterol-t as determined by several degradative procedures. The distribution of the tritium between the side chain and the tetracyclic rings is similar to that found by Fukushima and Gallagher (4) for deuterated cholesterol. Thus, 47.2 per cent of the tritium was located on carbon atoms 24, 25, 26 and 27. (The deuterated steroid had 52 per cent of the total radioactivity at these positions (4).) There was no tritium in the hydroxyl group because the cholesteryl chloride-t had the same specific activity as the cholesterol-t from which it was prepared. Positions 2, 3, 4 and 6 had 37.5 per cent of the tritium. (The deuterated cholesterol had 40 per cent and 3 per cent of the deuterium on carbon atoms 6 and 3, respectively (4).) Since the $C_{19}H_{30}$-t hydrocarbon and

TABLE 1. COUNTING ERRORS FOR TRITIUM AND CARBON-14 MIXTURES

Mixture prepared* DPM T 792 DPM C^{14} 342 Ratio $T/C^{14} = 3.26$

Found

T DPM	% Error	C^{14} DPM	% Error	Ratio T/C^{14}	% Error
736	7.1	244	0.4	2.77	15.0
831	4.9	266	9.5	3.09	5.2
903	14.0	266	9.5	3.39	4.0
996	25.7	269	10.7	4.08	25.2
Av. 886	Mean 12.9	Av. 261	Mean 7.5	Av. 3.31	Mean 12.4

Mixture prepared* DPM T 396 DPM C^{14} 121 Ratio $T/C^{14} = .327$

Found

T DPM	% Error	C^{14} DPM	T Error	Ratio T, C14	% Error
327	13.4	130	7.4	2.50	23.5
423	9.3	131	8.3	2.97	9.2
460	16.2	136	12.4	3.40	4.0
571	44.1	146	20.7	4.39	34.2
Av. 448	Mean 21.8	Av. 136	Mean 12.2	A. 3.31	Mean 17.7

* The same amounts of tritium and carbon-14 in four weighing bottles.

the methyl-3-β-Δ^5-cholenate-t had the same specific activity within the experimental error (Fig. 2), it was concluded that tritium was not stably bound at carbon atoms 20, 21, 22 and 23. About 16.2 per cent of the tritium in the rings was located at undetermined positions.

Table 2 shows the ratios of the tritium to carbon-14 specific activities of the plasma free cholesterol that was isolated during the ten-day period following administration of the tracers. The T/C^{14} ratio of the mixture of cholesterols that was administered was 8.0. The average value of the ratio for the blood cholesterol that was isolated was 7.0 ± 1.0. The 13 per cent discrepancy is probably not significant in view of the mean error of 12–17 per cent in determining the ratios of T/C^{14} (Table 1). These data indicate that cholesterol-t is stable in the blood for at least ten days. A similar conclusion was reached by Biggs and Kritchevsky (3) from studies of rabbits.

TABLE 2. STABILITY OF CHOLESTEROL-T *in vivo*

Time after administration of tracers (days)	Plasma free cholesterol		Ratio of specific activities T/C^{14}
	$\mu c.T/mM$	$\mu c.C^{14}/mM$	
0.17	0.44	0.65	6.7
0.33	0.24	0.031	7.7
0.50	0.30	0.034	8.8
2	0.19	0.029	6.6
3	0.30	0.053	5.7
4	0.26	0.039	6.8
6	0.20	0.025	8.0
8	0.12	0.017	6.8
10	0.12	0.019	6.3

The carbon-14 and tritium specific activities of the three C_{19} and two C_{21} urinary steroids that were isolated in this study are presented in Table 3. The ratios of the T/C^{14} specific activities are shown in the last column. The mean value for all the ratios was 4.0 ± 0.9.

By multiplying the percentage of tritium found in the rings of cholesterol-t (53.2 per cent) by the mean value of the T/C^{14} ratio of the plasma free cholesterol, one obtains 3.8, the value expected for the T/C^{14} ratio of the urinary steroids. If 8.0, the value of the ratio of specific activities of the

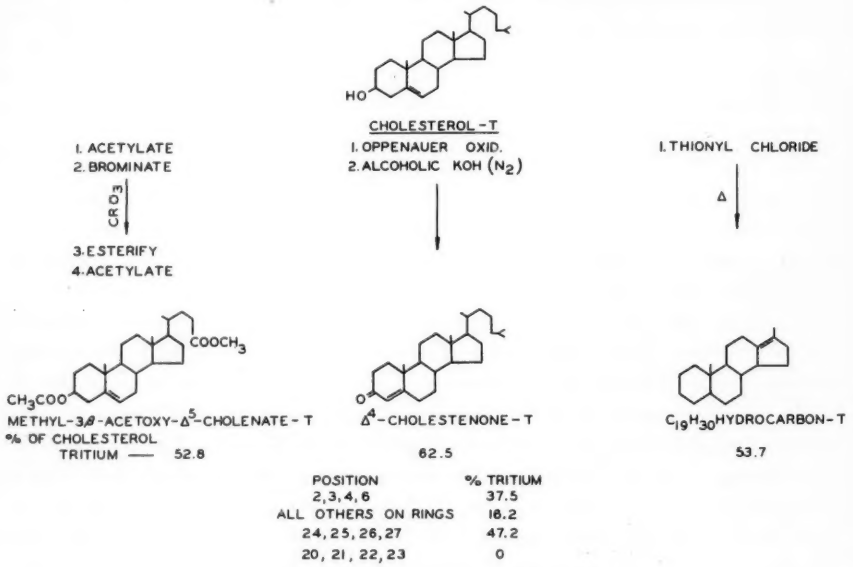


FIG. 2. Distribution of tritium in cholesterol-t.

TABLE 3. SPECIFIC ACTIVITIES OF CRYSTALLINE STEROIDS ISOLATED FROM THE URINE BY CARRIER TECHNIQUE

Day urine collected	Amt. isolated from urine (mg.)	Carrier added (mg.)	Steroid counted		Calculated by isotopic dilution		Ratio of specific activities T/C^{14}
			m.p.° C	mg.	μc.T/mM	μc.C^{14}/mM	
Androstan-3-α-ol-17-one							
1	1.75	17.59	186	10.77	0.11	0.21	5.2
4	1.24	10.44	185	8.30	0.089	0.035	2.5
5+6	2.40	7.91	186.5	1.82	0.10	0.029	3.5
7+8	1.50	10.14	186–187	6.38	0.072	0.022	3.4
Etiocholan-3-α-ol-17-one							
1+2	3.18	8.60	151–152	8.05	0.078	0.020	4.0
4	1.90	5.00	151–152	4.04	0.10	0.027	3.6
5+6	4.92	8.72	150–151	5.25	0.12	0.025	5.3
Etiocholan-3-α-ol-11,17-dione Acetate							
1+8	1.67	9.91	161–164	6.20	0.084	0.028	3.0
Pregnan-3-α-17-α-21-triol-11,20-dione Diacetate							
2	3.46	10.09	232–233.5	5.01	0.087	0.017	5.2
4	2.39	7.89	234	7.06	0.062	0.024	3.9
5+6	4.60	4.87	232.5	3.73	0.099	0.027	3.6
Pregnan-3-α-11-β-17-α-21-tetrol-20-one Diacetate							
2+6	1.1	—	209–217	1.1	0.11	0.021	5.2
	7.64	3.75	213–222	2.69	0.12	0.024	4.9

administered cholesterols, be used, then the expected ratio for the urinary steroids is 4.3. These calculations are based on the following assumptions: 1) that the conversion of cholesterol-t to urinary steroids involves no loss of tritium from the rings; 2) that the tritium from positions 24, 25, 26 and 27 on the side chain is not converted to urinary steroids to any appreciable extent; and 3) that the tetracyclic rings of 4-C^{14}-cholesterol are not degraded to smaller fragments, thereby releasing carbon-14 for possible steroid synthesis.[2]

The difference between the calculated values for the T/C^{14} ratio and that actually found in the urinary steroids was about 7.5 per cent. In view of the errors involved in determining the ratios, the agreement between the expected and found values is good and validates the assumptions made above.

[2] The authors are indebted to Dr. George Okita who assayed the expired air of the subject at intervals following administration of the labeled cholesterols. No $C^{14}O_2$ was detected. The lower limit of the assay was 1.8×10^{-6} μc./mM of $C^{14}O_2$. This indicates that degradation of the ring structure of carbon-14 cholesterol is unlikely.

The results of adrenal perfusion (18) and adrenal homogenate (19, 20) experiments have shown that cholesterol can serve as a precursor of cortisol. The data in Table 3 demonstrate that this conversion can take place *in vivo*, since cortisol has been isolated from adrenal vein blood (21, 22) and THE, THF and 11-ketoetiocholanolone are metabolites of this steroid (23).

Ungar and Dorfman (24) isolated carbon-14 androsterone and etiocholanolone from the urine of a female patient with an adrenocortical carcinoma who had been fed 3-C^{14}-cholesterol. The isolation of labeled androsterone and etiocholanolone in the present study suggests that under normal physiologic conditions, cholesterol is also a precursor for the 11-desoxy-C_{19}-steroids.

There is now ample evidence from tracer studies that adrenal steroids are derived from cholesterol, and from acetate. In the case of the latter precursor, it is not known whether adrenal steroids are synthesized directly from acetate or come from cholesterol produced from this precursor in the adrenal, the liver, or both. Experiments designed to elucidate this important problem are currently in progress.

SUMMARY

1. Tritium cholesterol is sufficiently stable to be useful for tracer studies of various aspects of cholesterol metabolism in man.

2. THE, THF, 11-ketoetiocholanolone, androsterone and etiocholanolone are derived in part from the intact tetracyclic rings of preformed plasma free cholesterol.

3. The side chain of cholesterol does not appear to be utilized for steroidogenesis.

4. In this study, about 53 per cent of the tritium was attached to the tetracyclic nucleus of cholesterol-t. The remainder was attached to the side chain.

Acknowledgments

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REGULATION OF SALT METABOLISM AFTER HYPOPHYSECTOMY IN MAN*†

JOHN P. MACLEAN, M.D., MORTIMER B. LIPSETT, M.D., MIN C. LI, M.D., CHARLES D. WEST, M.D. AND OLOF H. PEARSON, M.D.

The Division of Clinical Investigation, Sloan-Kettering Institute for Cancer Research and the Department of Medicine, Memorial Center, New York, N. Y.

THE control of sodium metabolism in the absence of the pituitary gland has not been clearly defined in man. Experiments in patients with panhypopituitarism have been inconclusive because of the variability of anterior pituitary function and the reliance upon inappropriate tests. We shall present our experiences with the hypophysectomized patient subjected to sodium restriction and shall show that sodium conservation remains efficient in the absence of the anterior pituitary gland.

There are decisive experimental data in animals (1) which demonstrate normal sodium metabolism after hypophysectomy. The secretion of aldosterone in response to sodium restriction and its relative independence of corticotropin (2) amply explain these findings. Aldosterone excretion has also been shown to be normal in the patient with panhypopituitarism (3) and to increase after hypophysectomy in response to sodium restriction (4). These considerations suggest that adrenal control of sodium metabolism is independent of pituitary function.

METHODS

These studies were conducted during the past two years on the Metabolic Ward of the James Ewing Hospital. All patients had had surgical hypophysectomy for metastatic cancer. Hypophysectomy was considered complete in each, on the basis of either complete suppression of the pituitary-dependent glands or the autopsy findings. All had diabetes insipidus. Substitution therapy varied and is listed separately.

Most of the patients were fed a constant diet from the Metabolic Kitchen. Sample diets were analyzed at the beginning and at two-week intervals throughout the study. Chemical methods in this laboratory have been previously described (5). The urinary

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17-hydroxycorticosteroids (17-OHCS) were measured by the method of Silber and Porter (6) and the blood 17-hydroxycorticosteroids (17-OHCS) as described by Nelson *et al.* (7). In the charts depicting balance data, the intakes are plotted down from the zero line; urine and fecal outputs are superimposed on the intake. Thus, shaded areas above the zero line represent negative balance.

RESULTS

Twelve studies of the response to a low-sodium diet were carried out in 9 patients. Figure 1 shows the response of a patient (D. W.) to sodium restriction. The rapid fall in urinary sodium and the early achievement of sodium balance are comparable to those found in normal subjects. The chloride balance paralleled the sodium balance. Complete balance data on Patient D. W. are presented in Table 1, and demonstrate that no other changes occurred throughout the period of salt withdrawal. These data are typical of those accumulated from studies in other patients subjected to the same regimen. It is further evident in this study that this patient could adjust to large sodium loads without excess retention.

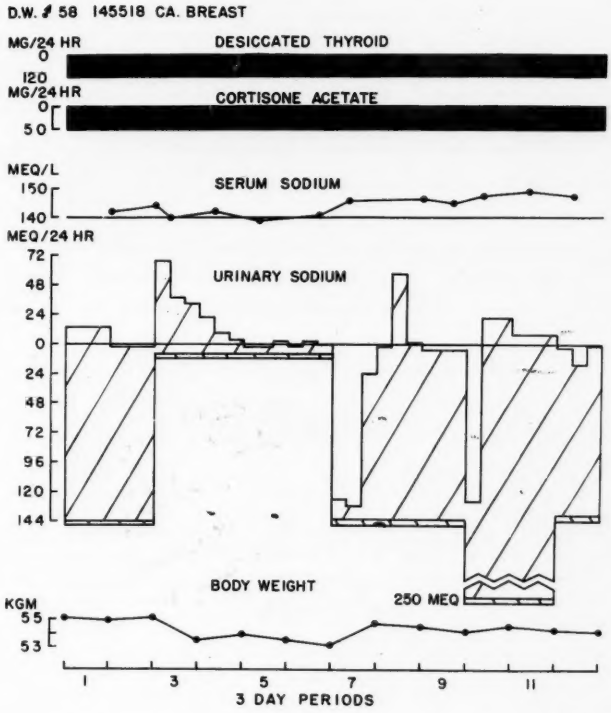

FIG. 1. Balance study with low-salt and high-salt intakes (*see* Table 1).

TABLE 1. COMPLETE BALANCE DATA ON HYPOPHYSECTOMIZED PATIENT D.W. ♂ CANCER OF THE BREAST (#145518)

Period	Day	Calcium (mg./24 hrs.)			Phosphorus (mg./24 hrs.)			Sodium (mEq./24 hrs.)			Chloride (mEq./24 hrs)		Nitrogen (Gm./24 hrs.)			Potassium (mEq./24 hrs.)			Creatine (Gm./24 hrs.)	Creatinine (Gm./24 hrs.)
		Urine	Feces	Balance	Urine	Feces	Balance	Urine	Feces	Balance	Urine	Balance	Urine	Feces	Balance	Urine	Feces	Balance		
Control (sodium intake 148 mEq./24 hrs.)	1	149.3	402.0	−311.6	696	490	−177	157.0	4.62	− 13.60	146	− 13.0	10.2	1.35	−0.25	49.6	16.40	+ 7.0	681	856
	2	149.3	402.0	−311.6	696	490	−177	157.0	4.62	− 13.60	146	− 13.0	10.2	1.35	−0.25	49.6	16.40	+ 7.0	681	856
	3	149.3	402.0	−311.6	696	490	−177	157.0	4.62	− 13.60	146	− 13.0	10.2	1.35	−0.25	49.6	16.40	+ 7.0	681	856
	4	148.3	402.0	−309.3	676	490	−157	141.3	4.62	+ 2.08	155	− 5.9	10.1	1.26	−0.15	51.6	16.40	+ 5.0	719	902
	5	148.3	402.0	−309.3	676	490	−157	141.3	4.62	+ 2.08	155	− 5.9	10.1	1.26	−0.15	51.6	16.50	+ 4.9	719	902
	6	148.3	402.0	−309.3	676	490	−157	141.3	4.62	+ 2.08	155	− 5.9	10.1	1.26	−0.15	51.6	16.50	+ 4.9	719	902
Low-salt (sodium intake 12 mEq./24 hrs.)	7	130.7	222.2	−112.0	661	257	+ 91	74.5	4.37	− 66.90	59.3	− 44.5	9.76	0.74	+0.79	36.2	8.69	+28.1	798	846
	8	130.7	222.2	−112.0	661	257	+ 91	44.4	4.37	− 36.77	59.3	− 44.5	10.10	0 74	+0.45	42.4	8.69	+21.9	798	846
	9	103.7	222.2	−112.0	670	257	+ 82	40.8	4.37	− 33.20	59.3	− 44.5	10.50	0.74	+0.05	53.9	8.69	+10.4	798	846
	10	117.3	222.2	− 99.0	716	257	+ 36	29.6	4.37	− 21.97	28.0	− 13.16	10.10	0.74	+0.45	52.9	8.69	+11.4	703	678
	11	117.3	222.2	− 99.0	724	257	+ 18	16.2	4.37	− 8.57	28.0	− 13.6	10.10	0.74	+0.45	52.9	8.69	+11.4	703	678
	12	117.3	222.2	− 99.0	640	319	+ 50	12.0	4.37	− 4.40	28.0	− 13.6	10.10	0.74	+0.45	52.9	8.69	+11.4	703	678
	13	107.1	253.6	−120.0	640	319	+ 50	5.52	3.32	+ 3.20	12.5	+ 2.28	9.52	2.16	−0.38	51.4	10.00	+11.6	594	720
	14	107.1	253.6	−120.0	640	319	+ 50	7.2	3.22	+ 1.50	12.5	+ 2.28	9.52	2.16	−0.38	47.5	10.00	+15.5	594	720
	15	107.1	253.6	−120.0	518	319	+172	12.2	3.32	− 3.50	12.5	+ 2.28	9.52	2.16	−0.38	48.6	10.00	+14.4	594	720
	16	96.2	253.6	−109.0	518	319	+172	7.7	3.32	+ 0.94	11.5	+ 3.28	9.00	2.16	+0.14	35.1	10.00	+36.9	532	605
	17	96.2	253.6	−109.0	518	319	+172	10.5	3.32	− 1.80	11.5	+ 3.28	9.00	2.16	+0.14	47.4	10.00	+15.6	532	605
	18	96.2	253.6	−109.0	518	319	+172	8.7	3.32	− 0.06	11.5	+ 3.28	9.00	2.16	+0.14	53.6	10.00	+ 9.4	532	605
High-salt (sodium intake 148 mEq./24 hrs.)	19	123.5	308.0	−192.0	662	426	− 79	16.8	5.22	+125.98	13.3	+135.40	10.00	1.34	−0.04	46.6	11.20	+15.2	490	734
	20	123.5	308.0	−192.0	654	426	− 71	11.9	5.22	+131.00	46.7	+102.00	9.74	1.34	+0.22	37.3	11.20	+24.5	648	868
	21	123.5	308.0	−192.0	567	426	+ 16	118.7	5.22	+ 24.00	112.0	+ 37.00	9.89	1.34	+0.07	45.5	11.20	+16.3	468	616
	22	133.0	308.0	−200.0	722	426	−139	141.2	5.22	+ 1.60	152.0	− 3.32	9.56	1.34	+0.40	41.9	11.20	+19.9	464	716
	23	133.0	308.0	−200.0	722	426	−139	200.2	5.22	− 57.00	152.0	− 3.32	9.56	1.34	+0.40	42.7	11.20	+19.1	464	716
	24	133.0	308.0	−200.0	722	426	−139	143.8	5.22	− 1.02	152.0	− 3.32	9.56	1.34	+0.40	59.0	11.20	+ 2.8	464	716
	25	106.5	308.0	−174.0	524	426	+ 59	104.4	5.22	+ 38.40	137.0	+ 11.68	9.01	1.34	+0.95	43.2	11.20	+18.6	344	683
	26	106.5	308.0	−174.0	524	426	+ 59	129.2	5.22	+ 14.60	137.0	+ 11.68	9.01	1.34	+0.95	48.7	11.20	+13.1	344	683
	27	106.5	308.0	−174.0	524	426	+ 59	180.0	5.22	− 38.00	137.0	+ 11.68	9.01	1.34	+0.95	51.8	11.20	+10.0	344	683
High-salt (sodium intake 250 mEq./24 hrs.)	28	127.5	308.0	−195.0	584	426	− 1	119.4	4.02	+126.60	204.0	+ 48.00	9.21	1.34	+0.75	40.4	11.20	+21.4	530	707
	29	127.5	308.0	−195.0	584	426	− 1	261.0	4.02	− 15.00	204.0	+ 48.00	9.21	1.34	+0.75	54.9	11.20	+12.9	530	707
	30	127.5	308.0	−195.0	584	426	− 1	273.1	4.02	− 27.00	204.0	+ 48.00	9.21	1.34	+0.75	50.6	11.20	+11.2	530	707
	31	135.6	308.0	−203.0	597	426	− 14	266.2	4.02	− 20.00	264.0	− 12.00	10.35	1.34	−0.39	60.7	11.20	+ 1.1	560	716
	32	135.6	308.0	−203.0	597	426	− 14	225.9	4.02	+ 20.00	264.0	− 12.00	10.35	1.34	−0.39	48.6	11.20	−13.2	560	176
	33	135.6	308.0	−203.0	597	426	− 14	269.0	4.02	− 23.00	264.0	− 12.00	10.35	1.34	−0.39	51.6	11.20	+10.2	560	716
	34	114.0	308.0	−181.0	551	426	+ 32	140.6	4.10	+ 3.30	148.0	+ 1.13	9.21	1.34	+0.75	30.5	11.20	+31.3	728	721
	35	114.0	308.0	−181.0	551	426	+ 32	127.2	4.10	+ 16.70	148.0	+ 1.13	9.21	1.34	+0.75	45.6	11.20	+16.2	728	721
	36	114.0	308.0	−181.0	551	426	+ 32	142.8	4.10	+ 1.10	148.0	+ 1.13	9.21	1.34	+0.75	50.0	11.20	+11.8	728	721

In Table 2 is a summary of similar studies in 7 other patients. The 2 patients whose intake is listed as low were fed the ward low-salt diet which contains, on the average, 15 mEq. of sodium daily. Again, the response to sodium restriction was within the normal range. The type of replacement therapy did not alter the response to sodium restriction. Parenthetically, it should be noted that there were no adverse symptoms or clinical effects from the salt restriction. The total exchangeable sodium was within normal limits whenever it was estimated. Glomerular filtration rate, measured by endogenous creatinine clearance, was unaltered by the salt restriction.

In view of the postulated relationship of potassium intake to aldosterone secretion (8), the data on Patient H. M. shown in Figure 2 are pertinent.

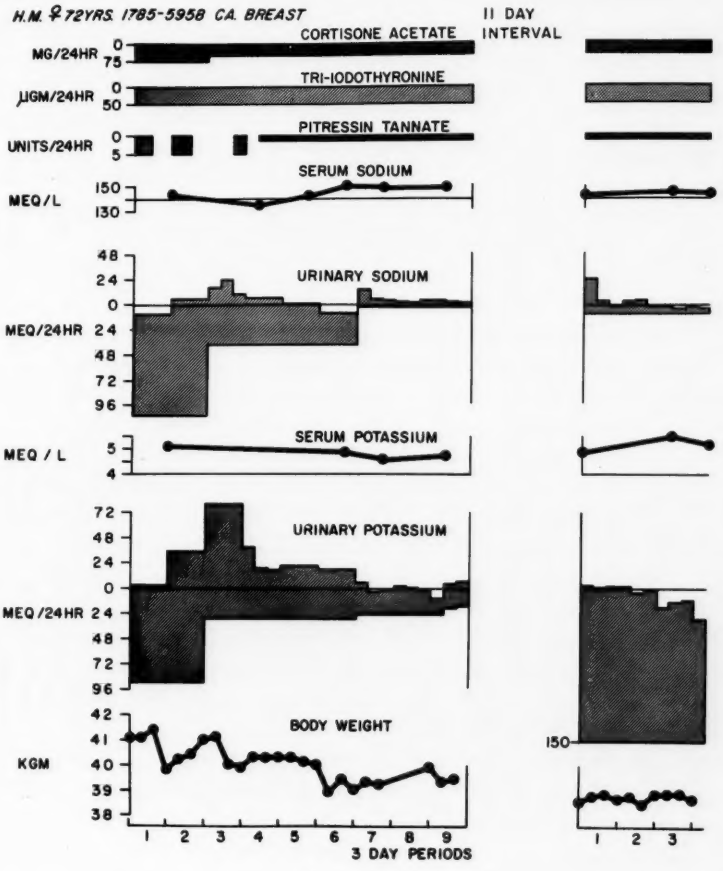

FIGURE 2

TABLE 2. RESPONSE TO SODIUM RESTRICTION IN 7 HYPOPHYSECTOMIZED PATIENTS

Patient	Medications			Severity of diabetes insipidus*	Na intake (mEq./24 hrs.)	Urine Na at end of salt restriction (mEq./24 hrs.)	Serum Na before Na restriction (mEq./24 hrs.)	Serum Na at end of Na restriction (mEq./L)	No. of days to reach Na balance	Total exchangeable Na (mEq./Kg. body wt.)
	Steroids	Thyroid	Pitressin							
I.M.	Cortisone, 25 mg. q8h	0	0	Mild	Low	8.0	146	148	7	—
J.M.	Cortisone, 25 mg. q12h	120 mg. qd	0	Mild	11.4	4.8	142	138	6	—
J.N.	Cortisone, 25 mg. q12h	0	0	Moderate	14.0	6.0	141	138	6	34.5
J.B.	Cortisone, 25 mg. q12h	0	0	Mild	10.0	3.7	141	140	20†	46.5
H.M.	Prednisone, 5 mg. q8h	Triiodothyronine, 75 μg. qd	0	Mild	10.4	1.2	143	144	5	44.8
H.M.	Prednisone, 5 mg. q8h	Triiodothyronine, 75 μg. qd	0	Mild	10.4	.93	137	141	15‡	—
F.H.	0	0	0	Moderate	Low§	5.0	141	140	5	—
K.K.	0	0	0	Moderate	6.5	5.8	140	136	6	37.3

* Mild = Urine output not more than 3500 ml./24 hrs.
Moderate = Urine output greater than 3500 ml. and less than 6000 ml./24 hrs.

† Marked edema of arm secondary to radical mastectomy. Edema receded during period of sodium loss, and then sodium equilibrium was attained.

‡ Pitting edema of ankles which receded before sodium equilibrium was achieved.

§ This patient was anoretic throughout the study and her sodium intake was less than 15 mEq./24 hrs.

Sodium and potassium balances were studied in a patient receiving 1 mEq. of sodium daily and potassium intakes of 30 mEq. and 145 mEq. daily respectively. A liquid formula diet (9) was used, supplemented with potassium chloride in the second part of the study. Sodium balance was obtained with equal facility during both high and low potassium intakes. The urine was analyzed for sodium-retaining factor by Dr. J. A. Leutscher, Jr., of Stanford University Medical School; this factor was found to increase fourfold under the low-sodium, low-potassium regimen. Thus, our study suggests that alterations in potassium intake or sodium-potassium ratio alone do not affect aldosterone secretion.

As administration of cortisone alone will permit some degree of sodium conservation in the adrenalectomized patient, 3 hypophysectomized patients were studied on a low sodium intake after withdrawal of cortisone. The significant data for 2 of them (F. H. and K. K.) are presented in Table 2 and demonstrate that sodium balance remained unchanged in the absence of cortical steroids.

Throughout the study, Patient F. H. was moderately ill due to lack of 17-OHCS. The urinary 17-ketosteroids reached a level of 1.7 mg. per twenty-four hours. Patient K. K., maintained on 5 mg. of prednisone every eight hours, was given a diet with an average salt content of 5.8 mEq. daily. Two days after prednisone was discontinued, the patient displayed signs and symptoms of severe adrenal insufficiency. Plasma corticoid concentration, after forty-eight hours of steroid withdrawal, was unmeasurable. During this period, the urinary sodium remained unchanged.

Patient F. N., whose data are charted in Figure 3, was maintained with Cortrophin-zinc,® which was then gradually withdrawn. After four days without corticotropin, urinary 17-OHCS had virtually disappeared but urinary sodium remained low. Moderate adrenal insufficiency was manifest at the end of five days, and corticotropin was again administered. These manipulations of the adrenal secretion of 17-OHCS affected neither the sodium balance nor, by inference, the secretion of aldosterone.

DISCUSSION

There are few systematic investigations of the adequacy of sodium conservation after loss of anterior pituitary function. Starr and Davis (10) employed the Cutler-Power-Wilder test (11) in 18 patients having some degree of panhypopituitarism, and showed that in 5 of them the chloride concentration in the final 4-hour urine specimen was above normal. They noted that the 2 patients with the highest chloride excretion were in excellent clinical condition, implying that there was no long-term negative sodium balance. Stephens (12) made a similar study in 7 patients with more severe hypopituitarism and demonstrated an abnormal response in 6 pa-

† Pitting edema of ankles which receded before sodium equilibrium was achieved.
§ This patient was anoretic throughout the study and her sodium intake was less than 15 mEq./24 hrs.

tients. In these cases the serum chloride level fell during the period of the test and symptoms such as weakness, nausea and headache were prominent.

Nurnberger and Korey (13) subjected 9 patients with chromophobe adenoma and hypopituitarism to salt deprivation and noted adverse symptoms or a decrease in serum sodium concentration in 4 patients. Significantly, they observed no diuresis in the patients with abnormal responses, which suggests only small renal losses of salt. Peters *et al.* (14) recorded their detailed studies of 34 patients with anterior pituitary hypo-

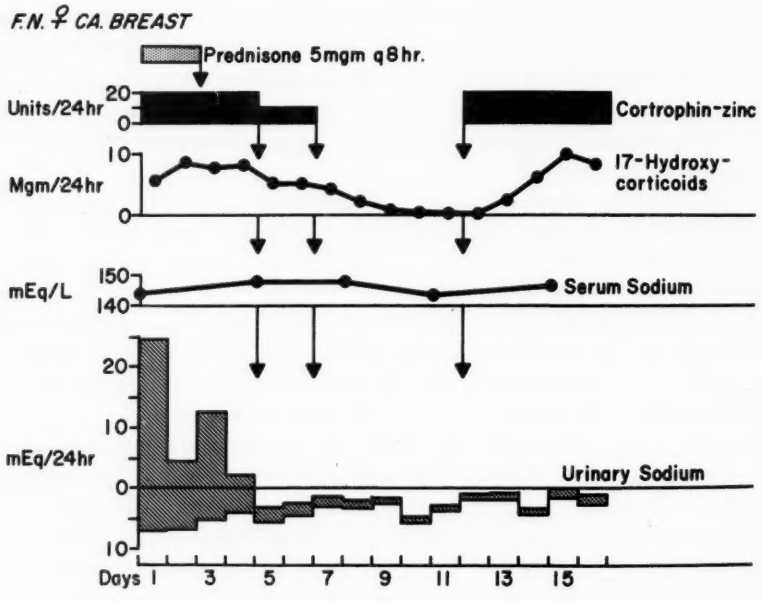

FIGURE 3

function. Hyponatremia was seen in association with surgical procedures and other stress, but in only 1 instance did hyponatremia result from salt restriction alone. In this case, it is not clear from the protocol if fluids were forced concurrently.

The abnormal results noted in the studies of these four groups of investigators have been offered as evidence that the adrenal mechanisms involved in sodium conservation may be impaired in the absence of the anterior pituitary gland. In order to resolve the apparent differences between the earlier studies and those presented in this paper, it should be recalled that the Cutler-Power-Wilder test includes both sodium restriction and a

regimen of forced fluids. In the absence of 17-OHCS, the excretion of water loads is greatly delayed (15), and mild water intoxication may ensue. It has also been pointed out (16) that the combination of relatively small electrolyte loss and mild water retention can cause severe hyponatremia and water intoxication. Thus, with the restriction of salt and the forcing of fluids in the Cutler-Power-Wilder test, conditions exist which lead to hyponatremia.

This clinical syndrome of water intoxication in patients with panhypopituitarism was studied by Wynn and Garrod (17). They described 2 patients with weakness, lethargy and hyponatremia. Both patients retained water in excess of electrolytes while they were receiving an unrestricted diet. When cortisone was given, there was a water diuresis and the serum sodium level rose, but the balance data showed only a small positive sodium balance. Had cortisone been unavailable, these cases would have been cited as examples of sodium depletion.

It has also recently become evident that water intoxication *per se*, may precipitate natriuresis and chloruresis. Leaf *et al.* (18) demonstrated that water retention caused by Pitressin led to increased excretion of sodium in the presence of a low level of serum sodium. Beck *et al.* (19) and Liddle *et al.* (20) have suggested that one component of this natriuresis is the suppression of aldosterone secretion when some moiety of the extracellular fluid is expanded. From these considerations, it appears that sodium depletion cannot be inferred from the combination of hyponatremia and natriuresis when the possibility of water retention exists.

In our patients, the demonstration of prompt reduction in urinary salt loss and maintenance of normal serum sodium levels in response to sodium restriction was attributable to two factors. First, most of the patients were receiving adrenal steroid replacement therapy, which precluded the possibility of water intoxication. Secondly, the presence of diabetes insipidus in all patients also tended to prevent water retention, even when steroids were withdrawn. The role of antidiuretic hormone in the genesis of the hyponatremia which results from cortisone withdrawal has been discussed (21), and it has been shown that the hyponatremia does not necessarily result from salt loss. A comparison of the effects of withdrawal of cortisone from adrenalectomized patients and from hypophysectomized patients with diabetes insipidus (22) further emphasizes this role of antidiuretic hormone. Therefore, although hyponatremia is not seen in the presence of diabetes insipidus, it is apparent that in panhypopituitarism without diabetes insipidus, and in the absence of steroids, hyponatremia may be a common sequela.

None of the present studies was carried out more than a year after hypophysectomy. It is therefore impossible to state that complete atrophy of

the adrenals will not occur in the absence of the anterior pituitary. However, as there are no unequivocal data which prove complete loss of adrenal function after many years of panhypopituitarism, our tentative conclusion is that sodium conservation remains independent of pituitary control.

SUMMARY AND CONCLUSIONS

1. The absence of the anterior pituitary gland does not modify the response to sodium restriction.

2. The reasons for the abnormal results obtained with the Cutler-Power-Wilder test in cases of panhypopituitarism are discussed.

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ADRENAL FUNCTION AFTER HYPOPHYSECTOMY IN MAN*†

MORTIMER B. LIPSETT, M.D., CHARLES D. WEST, M.D., JOHN P. MACLEAN, M.D. AND OLOF H. PEARSON, M.D.

The Division of Clinical Investigation, Sloan-Kettering Institute for Cancer Research and the Department of Medicine, Memorial Center, New York, N. Y.

ATROPHY of the adrenal gland following removal of the pituitary gland has been studied both functionally and anatomically in a variety of species but remains relatively unexplored in man. We have previously described some of the effects of total hypophysectomy on gonadal (1) and thyroid function (2) and propose now to examine several aspects of adrenal function after hypophysectomy.

It has been demonstrated that the secretion of aldosterone in man is independent of the anterior pituitary gland (3). This, however, carries no implications with respect to the secretion of hydrocortisone by the adrenal gland. In several species, hypophysectomy has been found to reduce markedly the secretion of corticosterone and hydrocortisone (4, 5, 6). The correspondence between animal and man of the responses to the other tropic hormones suggests that the secretion of hydrocortisone in man may be equally dependent upon corticotropin.

METHODS

These studies were conducted with patients who had had surgical hypophysectomy or adrenalectomy in an attempt to palliate metastatic cancer. Steroid withdrawals were usually undertaken within three months of hypophysectomy, after convalescence was complete, and as late as one year after adrenalectomy. Maintenance steroid therapy varied, and is discussed separately.

Complete metabolic balance studies were performed on the Metabolic Ward of the James Ewing Hospital. Constant diets were prepared in the Metabolic Kitchen and sample diets analyzed initially and at two-week

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intervals. Analytical methods of this laboratory have previously been described (3).

RESULTS

Generally the pattern of illness following withdrawal of cortisone or corticotropin (ACTH) from the hypophysectomized patient was like that seen in similar studies of adrenalectomized patients. In a variable number of days after withdrawal of steroids, anorexia, malaise and weakness ensue. These may progress to nausea and vomiting, and severe asthenia. Fever usually occurs and may reach 104° F. There is a variable drop in blood pressure, which may be severe and accompanied by postural hypotension.

In order to determine whether the hypophysectomized patient differs from the adrenalectomized patient, the clinical patterns of the withdrawal studies were compared between the two groups. The severity of the response to lack of 17-hydroxycorticosteroids (17-OHCS) was graded (Table 1). Those patients who were mildly ill and in whom there was no urgency about reinstituting cortisone therapy were classed as Grade 1. The other criteria are listed in Table 1. In this group, as well as in Grades 2 and 3, the response to cortisone was dramatic, consisting of return of appetite, sense of well-being and defervescence within twenty-four hours.

Table 1

CLINICAL EFFECTS OF WITHDRAWAL OF 17-HYDROXY-CORTICOSTEROIDS

	GRADE 1 Temp. <101° No change in B.P. Long Duration Mildly ill	GRADE 2 Temp. 101°-103° Drop in B.P. ± Duration 5-14 days Moderately ill	GRADE 3 Temp. >103° Drop in B.P. Duration <5 days Severely ill
ADRENALECTOMY	1 (7%)	11 (68%)	4 (25%)
HYPOPHYSECTOMY	7 (29%)	10 (42%)	7 (29%)

Patients who were classed in Grade 3 became severely ill in a short period of time after withdrawal of therapy. In these cases we were forced to replace cortisone because of rapid clinical deterioration of the patient.

In the intermediate group (Grade 2) were the patients who, in our judgment, would have become critically ill if any stress had been added. However, within the protective environment of the hospital, these patients were

able to survive for longer periods, although they remained weak, febrile and anoretic throughout.

Each patient did not necessarily display all the criteria listed for each grade, and occasionally the grade of the withdrawal symptoms had to be assigned on the basis of a majority of the criteria. In Table 1, the type of response to 17-OHCS withdrawal is classified for the adrenalectomized and hypophysectomized patients. It may be significant that in the only adrenalectomized patient in Grade 1, there existed collateral evidence that residual functioning adrenal tissue remained. If this case is discarded, then

TABLE 2. CHARACTERISTICS OF HYPOPHYSECTOMIZED PATIENTS WITH MILDEST RESPONSE TO STEROID WITHDRAWAL (GRADE 1)

Patient	Urinary gonadotropins (m.u./24 hrs.)	Serum protein-bound iodine (μg./100 cc.)	Thyroidal 48-hr. I^{131} uptake (% dose)	Lowest 17-KS during withdrawal (mg./24 hrs.)	Autopsy data
I.M.	0	1.2	2.6	1.1	Total hypophysectomy
M.P.	0	2.8	32	1.9	
H.A.	0	—	1.9	1.0	Total hypophysectomy
I.R.	0	1.5	9.6	1.5	
M.R.	0	0.6	4.9	0.5	Total hypophysectomy
F.H.	0	0.2	5.6	1.7	
A.H.	0	—	10.7	Plasma 17-OHCS, 0.26 μg./100 cc. Urine 17-OHCS, 0.46 mg./24 hrs.	

the difference in severity of withdrawal reactions is statistically significant by the Chi-square test ($P<.05$). Apart from statistical considerations, however, it is pertinent to examine closely the 7 hypophysectomized patients who became only mildly ill after withdrawal of 17-OHCS.

Data indicating the completeness of the hypophysectomy in these 7 patients are listed in Table 2. Autopsies were performed in 3 instances, and serial sections of the sella turcica did not disclose any pituitary remnants. There was no pituitary gonadotropin in the urine, and thyroid function was severely depressed. In 6 patients, urinary 17-ketosteroids were below 2.0 mg. per twenty-four hours. In the seventh patient, plasma 17-OHCS levels were low at the end of the withdrawal period, and urinary 17-OHCS

was 0.46 mg. per twenty-four hours. Thus, none of the patients classified Grade 1 had evidence of either incomplete hypophysectomy or persistent adrenal function.

Half of the hypophysectomized patients were studied during steroid withdrawal under conditions of either a complete balance regimen or a sodium and potassium balance regimen. These studies contributed little towards an understanding of adrenal insufficiency, as there were essentially no changes. The salt intake was not kept high, as in the studies of the adrenalectomized patients, because the continued secretion of aldosterone (3) ensured maintenance of sodium balance.

The glomerular filtration rate, which was estimated by endogenous creatinine clearance, fell in each case studied during steroid withdrawal. Fasting blood sugar values after steroid withdrawal were unchanged in 12 patients and dropped 15, 20 and 35 mg. per 100 cc. respectively in 3 patients. In none of these patients did clinical hypoglycemia develop. Serum potassium levels were unaltered. The absolute eosinophil count was significantly higher in 3 of 17 patients studied. The lowest 24-hour 17-ketosteroid excretion during withdrawal averaged 3.3 mg. in the hypophysectomized patients—a value not significantly different from the average of 2.6 mg. per twenty-four hours found in the adrenalectomized patients not receiving steroid replacement.

These essentially minor changes are like those observed in adrenalectomized patients during steroid withdrawal. The one marked difference was in the level of serum sodium. It has been demonstrated that the serum sodium concentration often falls during an adrenal crisis, in spite of no external sodium loss (7). The hypophysectomized patients, however, rarely displayed hyponatremia during adrenal insufficiency. Figure 1 emphasizes this marked difference and suggests the correlation with the presence of diabetes insipidus. Of the adrenalectomized group, only 5 patients did not have a drop in the serum sodium level of 9 mEq. or more, and 2 of these patients had diabetes insipidus. On the other hand, only 4 of the hypophysectomized patients demonstrated hyponatremia of like degree. Two of these 4 patients were receiving Pitressin® and one had a normal water exchange. Thus, with few exceptions, the presence of even mild diabetes insipidus prevented the development of hyponatremia which occurs after withdrawal of steroids.

It was conceivable that the continued secretion of aldosterone in the hypophysectomized patients ameliorated the effects of lack of 17-OHCS. Therefore, 5 patients were subjected to severe sodium restriction, and after sodium balance was achieved, the corticotropin or steroid replacement was discontinued. In each case, typical adrenal insufficiency developed, seemingly unaltered by the increased secretion of aldosterone. Figure 3 of the

preceding paper (3) is an example of this type of study and illustrates that throughout the period of developing adrenal insufficiency, the urinary sodium remained low, implying the continued secretion of aldosterone. We tested the effects of cortisone withdrawal in 1 patient while she was receiving first a normal salt intake and then a low salt intake, and could detect no differences in the rate of development or severity of adrenal crisis.

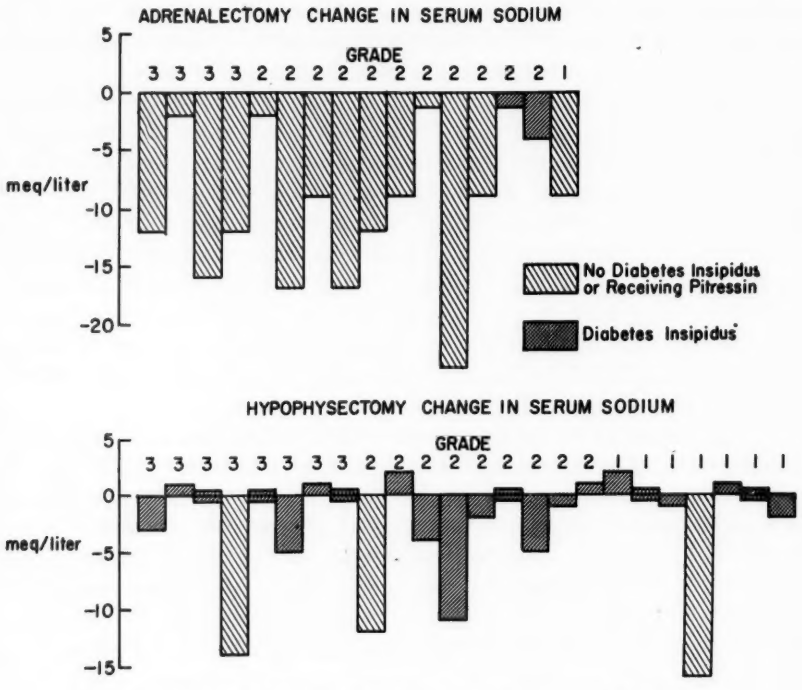

FIG. 1. Effect of withdrawal of 17-hydroxycorticosteroid therapy on serum sodium level.

It, therefore, seemed that aldosterone in physiologic amounts could not protect against adrenal crisis due to lack of 17-OHCS.

We subsequently studied the effects of exogenous aldosterone in 2 adrenalectomized patients. In one, 900 micrograms of aldosterone given intramuscularly over a 36-hour period, did not alter the development of adrenal crisis, whereas an identical study in the other patient yielded opposite results. This amount of aldosterone is apparently well beyond the physiologic range of secretion, which may account for the different responses. It is, nevertheless, somewhat surprising that aldosterone in this dosage was

effective in one of the patients, as the cortisone-like activity of aldosterone is not too different from the activity of cortisone on a weight basis.

DISCUSSION

Although we have not proved that the effect of steroid withdrawal after hypophysectomy is milder than after adrenalectomy, the data of Table 1 are suggestive. It may be worthwhile, therefore, to consider the factors which are different between the two groups and that might cause an amelioration of the induced adrenal insufficiency.

The secretion of aldosterone by the hypophysectomized patient would not seem to be a significant factor. The inability of desoxycorticosterone to maintain life in the adrenalectomized patient is the counterpart of the inability of aldosterone to prevent adrenal insufficiency. Many investigators (8, 9) have maintained Addisonian patients with aldosterone, but many Addisonian patients may be maintained with desoxycorticosterone alone. The activity of aldosterone, estimated by parameters other than sodium retention and life maintenance in animals, is relatively low (10) and consistent with the hypothesis that its physiologic role is concerned with the conservation of salt at low levels of intake. Thus, the evidence from the literature does not conflict with our view that the secretion of aldosterone does not modify the effects of 17-OHCS deficiency.

Any differences in withdrawal patterns between the two groups could not be attributed to change in sodium balance, as sodium balance was maintained in the adrenalectomized patients with supplemental salt and in the hypophysectomized patients by endogenous aldosterone.

The presence of diabetes insipidus, *per se*, exerted no effect on the development of adrenal insufficiency. Adrenal crisis occurred as readily in patients with severe diabetes insipidus as in those without diabetes insipidus. A relevant study was performed by Winter *et al.* (11), who noted that adrenalectomized cats with diabetes insipidus died sooner than adrenalectomized cats when adrenocortical extract was stopped, although the serum sodium level remained normal.

As many of our studies were performed within the first two months after hypophysectomy, the patients were not receiving thyroid replacement medication. Their status generally was that of chemical hypothyroidism, with only an occasional patient displaying clinical hypothyroidism. Peterson and co-workers (12) have shown that the disappearance rate of hydrocortisone is slower in the hypothyroid patient. It is therefore possible that depressed thyroid function in the hypophysectomized group may have contributed to the lesser severity of the withdrawal reactions.

Other possibilities exist, however, which are not ruled out by our studies. The continued secretion of small amounts of 17-OHCS below the level of

detection of the present methods cannot be ignored. The totally hypophysectomized dog (4), rat (5) and guinea pig (6) continue to secrete small amounts of 17-OHCS in the presumed absence of corticotropin, so that precedent for this hypothesis exists in several species.

A nasopharyngeal pituitary gland has been described (13), and although its functional status is unknown, its possible role should not be ignored.

The explanation, then, of the differences in withdrawal patterns is obscure at present, but several hypotheses have been suggested which are susceptible to experimental testing.

The comparison of the results of 17-OHCS withdrawal between adrenalectomized and hypophysectomized patients illuminates a finding of physiologic significance. The occurrence of hyponatremia in the adrenalectomized patient maintained in sodium balance during steroid withdrawal has been variously attributed to shifts of water and electrolytes (7, 14). It has been suggested (7) that water intoxication was of some significance in this regard. This suggestion receives strong support from the data of Figure 1, which clearly demonstrate the importance of adequate amounts of antidiuretic hormone in the development of hyponatremia. It is not necessary, however, to postulate decreased disposal of antidiuretic hormone but only that its presence is essential for the dilution hyponatremia. Such a mechanism does not rule out postulated fluid and electrolyte shifts; but in view of the great difficulties attendant upon interpreting small changes in volumes of distribution, this effect of antidiuretic hormone may be the only clearly established mechanism.

SUMMARY

1. Cortisone or corticotropin was withdrawn from totally hypophysectomized patients and certain characteristics of the resulting adrenal insufficiency have been studied. When compared with similar studies of adrenalectomized patients, the pattern of steroid withdrawal symptoms tended to be milder. Possible reasons for this are discussed.

2. Aldosterone in physiologic amounts does not alter the pattern of adrenal insufficiency resulting from a lack of 17-hydroxycorticosteroids.

3. The presence of antidiuretic hormone is necessary for the development of the hyponatremia associated with deficiency of 17-hydroxycorticosteroids in the absence of external salt loss.

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METABOLIC BALANCE STUDY IN A CASE OF VIRILIZING ADRENAL HYPERPLASIA TREATED WITH PREDNISONE*

WILLIAM A. SKOOG, M.D. AND WILLIAM S. ADAMS, M.D.

The Department of Medicine, School of Medicine, University of California Medical Center, Los Angeles, California

VIRILISM due to bilateral adrenal hyperplasia, although a relatively rare condition, has been the subject of considerable interest and research. Wilkins and associates (1–5) first reported the use of cortisone and related compounds in depressing excessive urinary 17-ketosteroid excretion in patients with the adrenogenital syndrome. Regression of the characteristic virilizing features of the syndrome is often associated with the fall in 17-ketosteroid excretion. Others (6–11) have confirmed these observations. Although much has been done in an attempt to understand the various aspects of this form of virilism, there have been no reports of long-term metabolic balance studies in patients receiving steroid therapy. The present study was undertaken in order to evaluate, under strict metabolic balance conditions, the treatment of a case of virilizing adrenal hyperplasia with one of the newer steroids, prednisone (Meticorten).

CLINICAL SUMMARY

P. V. was a 29-year-old white housewife referred to the UCLA Medical Center by Dr. William F. Walsh of Camarillo, California. Her chief complaint was hirsutism of sixteen years' duration. Excessive hair growth involving the face, legs, and arms was first noticed at the age of 9 and had increased so that by her late teens she found it necessary to shave the beard area daily. Male muscular development was also a prominent feature of her illness. Her menstrual periods began at age 10 and had been irregular since age 16. In recent years she had usually found it necessary to take ½ grain of thyroid daily in order to menstruate at all. She reached her maximum height at age 15. Greying of the hair began at this age, although premature greying was not a familial characteristic. At age 21, an appendectomy was performed and palpation of the adrenal areas at the time of operation revealed no definite abnormalities. An ovarian biopsy showed only small fibromatous surface nodules. She married at age 22 and a year and a half later lost a 3-month-old fetus.

On initial examination she was found to have profuse hypertrichosis. The hair of the extremities and of the beard, axillary and pubic areas was more extensive than that of the average male. In addition, there was a male type of muscular development.

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Her voice was feminine and she had normal genitalia without clitoral enlargement. There was no palpable mammary tissue in her breasts. Her blood pressure was normal.

The initial urinary 17-ketosteroid values were 40.5, 39.5 and 39.2 mg. per twenty-four hours (normal, 7.0–14.0 mg.). Urinary 17-hydroxycorticosteroids were 9.1 and 12.0 mg. per twenty-four hours (normal, 4.0–10.0 mg.). Urinary dehydroisoandrosterone was 2.2 mg. per twenty-four hours (normal, 2.2–5.0 mg.). The titer of urinary follicle-stimulating hormone was greater than 10 and less than 20 mouse units per twenty-four hours (normal, greater than 6 and less than 50 mouse units). Urinary pregnanediol and pregnanetriol values were normal, being 1.6 and 0.12 mg. per twenty-four hours, respectively. Roentgenograms of the skull and chest were normal. Intravenous pyelography and retroperitoneal pneumography revealed nothing to suggest a suprarenal mass. Other studies, including a glucose tolerance test, liver function tests, and determinations of the serum protein-bound iodine and blood urea nitrogen levels yielded normal results.

On the basis of the clinical and laboratory findings it was felt that the patient had adrenal hyperplasia with virilism. An 80-day metabolic balance study was undertaken in order to evaluate the effect of prednisone in the treatment of this condition. Prednisone (given in the amount of 10 mg. per day in divided dosage) was found to be effective in suppressing the excessive virilizing activity of this patient's adrenal glands. Following the metabolic balance study, she continued to receive prednisone and was seen at monthly intervals as an out-patient.

At the time of submission of this paper for publication she had been under observation for twelve months. There had been gradual improvement during this time, manifested principally by a more female body contour, by decreased muscular strength and, to a lesser extent, by diminution in hirsutism. She was two months pregnant. Unfortunately her beard hair was not weighed prior to therapy, but a four-day growth weighed 172 mg. after she had been treated for one and one half months. A comparable growth after six months of continuous treatment with prednisone in a dosage of 10 mg. per day, weighed 141 mg.—a difference of 31 mg. Her menstrual periods had been regular, without the administration of exogenous thyroid. Vaginal smears made at intervals throughout the study showed that, cytologically, the estrogen level was no different from that indicated in control smears. No complications or undesirable effects of steroid therapy had appeared.

Figure 1 shows the patient before and after seven months of continuous prednisone administration.

PROCEDURE AND METHODS

The patient studied was admitted to the Metabolic Unit where she remained under continuous observation. Her urine was analyzed daily for sodium, potassium, phosphorus, chloride, uric acid and nitrogen. Aliquots of the 24-hour collections of urine were saved and pooled in periods of five days, and the analyses repeated on the pooled samples. This served as an added check on the accuracy of the daily work. Stools were separated at intervals of five days (with the use of carmine markers) and analyzed for the same constituents as in the case of urine, with the exception of uric acid. Sample diets, duplicating the menu of the patient, were prepared at intervals of five days, homogenized, sampled, and analyzed to determine intake. Although a dietary rejection occurred on only one day during the study, it was treated in the same manner as the sample diet, analyzed separately, and appropriate corrections of intake made. Blood was collected in the postabsorptive state from an arm vein, with the least possible stasis. After prompt separation of the serum, determinations were made of sodium, chloride, potassium,

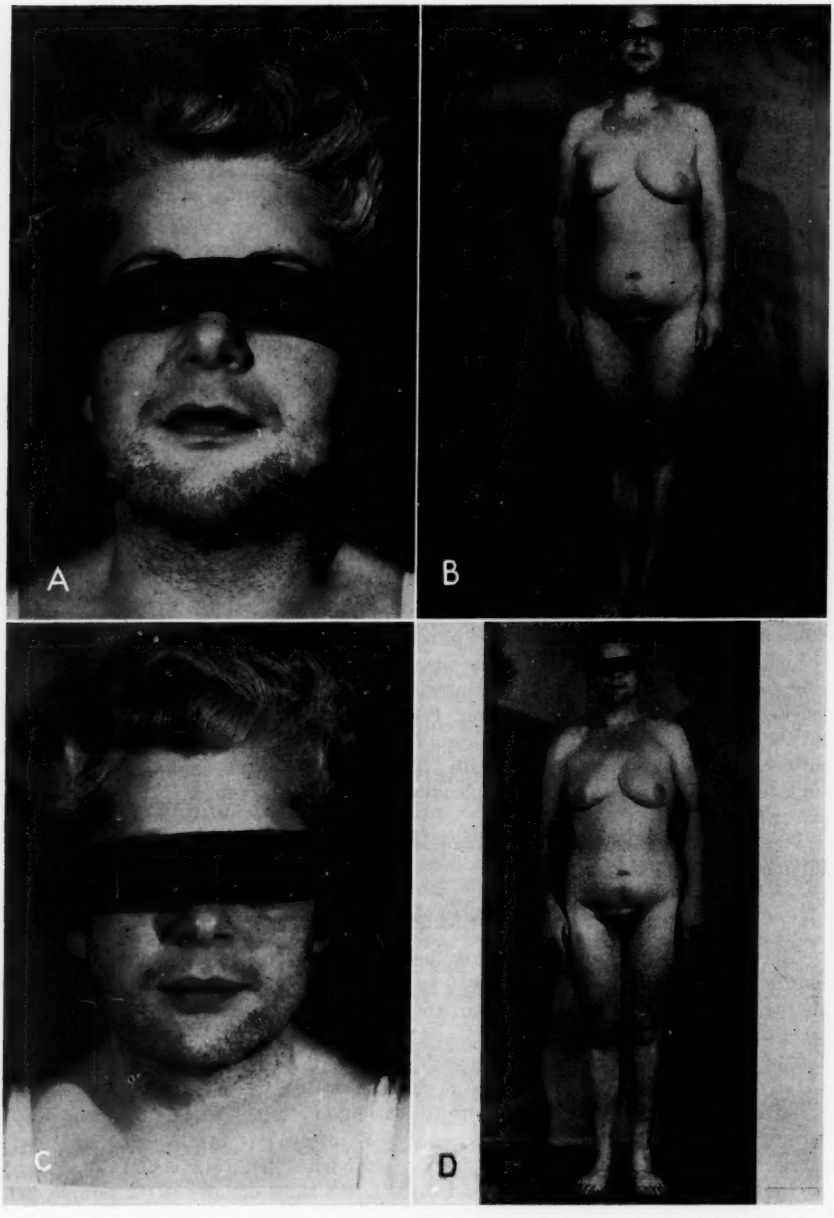

FIG. 1. *Patient P. V.* The upper two photographs were taken on May 27, 1955, prior to prednisone therapy; the lower two were taken on January 12, 1956, after seven months of continuous prednisone therapy. Each photograph was taken when the patient had a 4-day growth of beard.

calcium, phosphorus and uric acid. The analytical methods used were as follows: Aliquots of diet and urine were ashed in a muffle furnace at a temperature not exceeding 450° C. Sodium and potassium determinations were performed directly on diluted specimens of urine and (blood) serum, with the use of the Beckman flame photometer. With the exception of nitrogen and chloride, the other substances were determined from the ashed filtrates. Urinary, fecal and dietary calcium were determined by the gravimetric method of Washburn and Shear (12), and the level of serum calcium by the method of Van Slyke and Sendroy (13), phosphorus by the gravimetric method of Washburn and Shear (12) and a modification of the colorimetric method of Fiske and SubbaRow (14), nitrogen by the Hiller, Plazin and Van Slyke modifications of the Kjeldahl procedure (15), chlorides by the Sanderson potentiometric method (16), and serum and urinary uric acid by modification of the methods of Kalckar (17) and Praetorius (18). Urinary 17-ketosteroids were determined by a modification of the Zimmermann method (19), 17-hydroxycorticosteroids by the method of Reddy (20), pregnanediol and pregnanetriol by the method of Bongiovanni and Clayton (21), and dehydroisoandrosterone by the method of Gardner and Migeon (22).

METABOLIC STUDIES

Figures 2 to 4 depict the metabolic balance data. The values are charted according to a conventional method (23) of representing such data. The fluid intake was constant throughout the study. A period of twenty days was allowed for collection of control balance data. Then metabolic balances were determined during: 1) thirty days of prednisone in a dosage of 20 mg. per day (5 mg. every six hours); 2) twenty days of prednisone in a dosage of 10 mg. per day (5 mg. every twelve hours); and 3) a final ten days without prednisone therapy.

A. *Nitrogen, calcium and phosphorus balances*

During administration of 20 mg. of prednisone per day there was a moderately negative nitrogen balance accompanied by a 7-pound weight loss. During administration of 10 mg. daily, however, there was a return to the positive nitrogen balance of the control period, and no further weight loss. No significant changes in calcium were noted, the balance remaining positive throughout the study. Phosphorus excretion was only slightly increased during the higher dosages of prednisone. The excessive excretion of nitrogen and phosphorus on the fourth day after prednisone administration occurred in association with a large urinary output.

B. *Chloride, sodium and potassium balances*

It may be seen that the sodium and chloride balances mirror each other. The initial effect of prednisone given in a dosage of 20 mg. per day was to bring about a moderate sodium diuresis. This was gradually replaced by a fairly consistent positive sodium balance during the last week of this higher dosage, and this positive balance continued throughout the period of the

TABLE 1. NITROGEN, CALCIUM, PHOSPHORUS AND ELECTROLYTE BALANCES*

Period (5 days ea.)	Therapy (daily)	Balances						Body weight (lbs.)
		Ca (Gm.)	P (Gm.)	N (Gm.)	Na (mEq.)	K (mEq.)	Cl (mEq.)	
I	Control	0.815	0.318	3.0	117.8	34.0	137.7	187.6
II	Control	0.818	0.755	4.5	26.2	13.2	63.9	187.5
III	Control	0.699	0.327	6.0	50.6	25.9	73.0	187.7
IV	Control	0.744	0.871	15.5	100.0	48.2	236.2	187.8
V	Prednisone, 20 mg.	0.539	−1.607	−11.2	−191.8	−111.2	−132.1	187.0
VI	Prednisone, 20 mg.	0.516	0.003	− 5.1	− 37.6	17.2	47.3	185.8
VII	Prednisone, 20 mg.	0.477	0.163	− 5.6	− 21.7	11.1	-41.5	184.7
VIII	Prednisone, 20 mg.	0.571	−0.224	−12.1	− 31.5	− 21.9	37.3	183.3
IX	Prednisone, 20 mg.	0.943	−0.045	−12.8	− 22.0	− 36.6	29.1	182.4
X	Prednisone, 20 mg.	0.607	0.160	−10.4	20.6	− 16.7	21.2	181.6
XI	Prednisone, 10 mg.	0.590	−0.051	− 2.9	65.7	3.4	69.6	181.6
XII	Prednisone, 10 mg.	0.469	0.370	4.0	9.8	− 15.2	− 6.7	181.5
XIII	Prednisone, 10 mg.	0.678	1.004	10.0	202.4	73.2	125.2	180.9
XIV	Prednisone, 10 mg.	0.736	0.586	3.8	38.9	8.4	− 0.6	181.3
XV	Control	0.876	0.821	8.5	113.5	44.7	109.5	182.0
XVI	Control	0.789	1.361	16.2	− 3.9	80.7	− 12.9	183.0

* In computation of these balances, average values for dietary intake and fecal excretion were employed (actual analyses of both diet and stool having been carried out every five-day period). The diet was calculated to contain 103.6 Gm. of protein, 124.8 Gm. of fat, 224.9 Gm. of carbohydrate and 2,437 calories per day. The average values per five-day period were as follows:

	Diet	*Fecal excretion*
Calcium (Gm.)	5.733	3.8
Phosphorus (Gm.)	9.453	2.79
Nitrogen (Gm.)	89.0	5.0
Sodium (mEq.)	726.5	5.5
Potassium (mEq.)	477.0	38.0
Chloride (mEq.)	737.5	12.0

lower dosage of 10 mg. per day. Serum sodium values were moderately high, approaching 160 mEq. per liter during the control period and the first fifteen days of therapy, with a fall to lower levels during continued therapy. There was a slightly negative potassium balance in association with the higher dosage but not with the lower dosage of prednisone. Again, excessive excretion of these substances occurred on the fourth day after prednisone was started.

C. *Uric acid excretion*

No significant changes in uric acid excretion were noted, with the possible exception of the excessive urinary excretion seen on the fourth day after steroid administration was begun.

D. *17-ketosteroid excretion*

The 24-hour urinary excretion of 17-ketosteroid was determined at intervals of approximately every other day throughout the study. Normal values of 17-ketosteroids were observed after seven days of therapy. There was a slight increase in excretion initially during the lower dosage of prednisone, but the value again gradually fell to low-normal. When the steroid was withdrawn the 17-ketosteroid excretion gradually rose—and precipi-

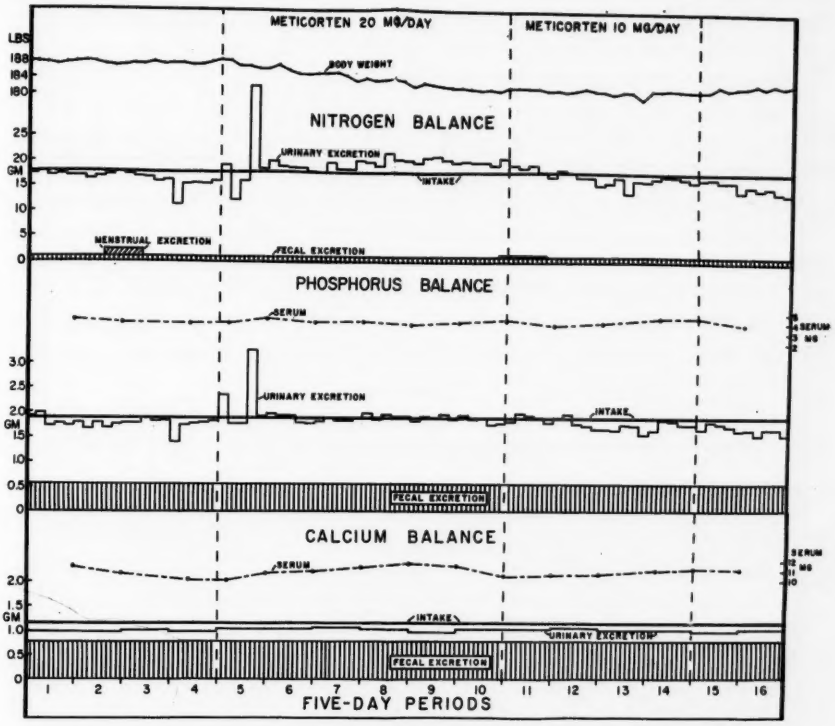

FIG. 2. Effect of prednisone (Meticorten) on nitrogen, phosphorus and calcium balances. The heavy horizontal dietary lines represent the average values for the dietary intake, determined once during each five-day period. The vertically hatched areas represent the average of fecal excretion values, determined for each five-day fecal pool. It was considered justified to average these results because only minor variations were observed in the five-day periods. Positive balances are indicated by clear areas between the intake line and the sum of urinary and fecal excretions. An extension of the line labeled "urinary excretion" above the line labeled "intake" denotes a negative balance.

tously so, when the adrenals were stimulated by 25 units of corticotropin administered intravenously over an 8-hour period. Urinary 17-hydroxycorticosteroids also rose markedly, providing further evidence of adrenal responsiveness. Urinary 17-ketosteroids, determined at intervals during the ten-month follow-up period, continued within normal limits.

DISCUSSION

Mechanisms and diagnostic considerations

The administration of cortisone or hydrocortisone to patients with adrenal hyperplasia and virilism results in a fall in urinary 17-ketosteroids. It is thought that this is the result of inhibition of corticotropin (ACTH)

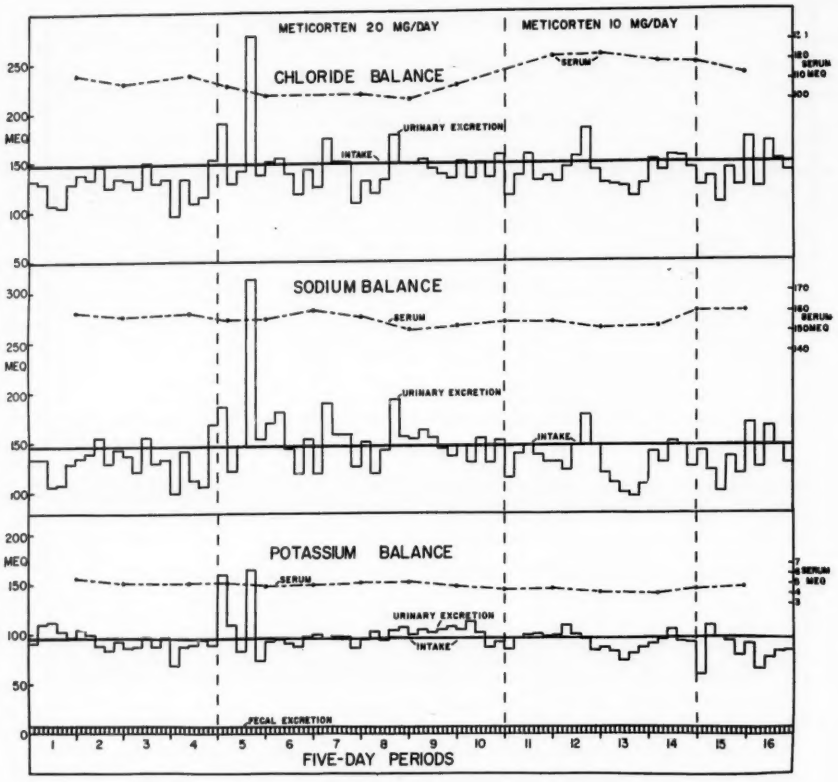

FIG. 3. Effect of prednisone (Meticorten) on sodium, chloride and potassium balances. (For key, *see* legend to Figure 2.)

production by the pituitary. Several workers (6, 24, 25) postulated that in this syndrome there is impairment of the ability of the adrenal cortices to synthesize gluconeogenic hormone (compound F) from its precursors. The decreased output of compound F leads to an increased production of corticotropin by the anterior pituitary. The defect in synthesizing compound F has been thought to be a result of an inability to hydroxylate simpler steroid intermediates at positions C-11, C-17 or C-21. Evidence has accumulated in support of this concept (26–28). Jailer *et al.* (29) hypothesized that the defects in hydroxylation mechanisms cause a piling up of partially hydroxylated hydrocortisone precursors which are metabolized along well known pathways, resulting in the production of androgens. These substances fail to inhibit the production of pituitary corticotropin adequately, thus permitting an increase in circulating corticotropin (30) and resultant

adrenal hyperplasia. In studies of the metabolism of adrenal steroids in the adrenogenital syndrome (31), Bongiovanni and associates indicated that pregnanetriol is regularly present in large quantities in the urine of untreated patients. They postulated that this represents a block in the synthesis of compound F at some point between the latter and its precursor, 17-hydroxyprogesterone. The lack of increased excretion of pregnanetriol in our patient is unusual, but may indicate that a less common defect or combination of defects in the synthesis of compound F was present. It is possible that the defect is similar to that recently reported in a case of compound S-secreting hyperplasia of the adrenals by the same authors (32). The findings in that patient strongly suggested an essentially isolated and complete deficiency of the adrenal enzyme(s) concerned with the hy-

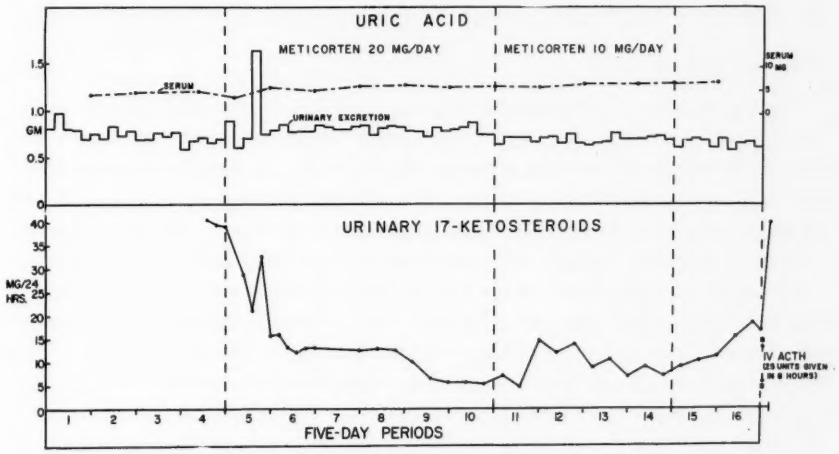

FIG. 4. Effect of prednisone (Meticorten) on serum uric acid level and on urinary uric acid and 17-ketosteroid excretions.

droxylation of the steroid molecule at C-11. Excessive excretion of pregnanetriol was not demonstrated. Our patient's normal excretion of dehydroisoandrosterone, known to be excreted in large amounts by persons with adrenal tumors (33–36), provided additional evidence that the diagnosis of adrenal hyperplasia was the most tenable one.

Most patients with virilizing syndromes related to adrenal hyperplasia or tumor have, in addition to hirsutism, other manifestations of androgenicity such as deepening of the voice and enlargement of the clitoris. In our patient, however, except for male muscular development, these signs were not present. Kinsell and Lisser's (37) patient with a large adrenocortical tumor demonstrated only heterosexual hypertrichosis and a tendency to

increased skeletal musculature. They postulated that the adrenal tumor in this case was excreting an excessive amount of the anabolic steroid normally manufactured by the postpubertal adrenal cortex. Kinsell has suggested (38) that our patient's hyperplastic adrenals may have been excreting the same sort of anabolic steroid. It would appear profitable to investigate this possibility further in our patient.

Response to therapy

The response to therapy exhibited by our patient is consistent with responses observed by Wilkins *et al.* (3, 39). They stated that the clinical effects observed during continuous cortisone therapy depend on the degree of virilization attained before treatment is instituted. The suppressive dose of prednisone found satisfactory in our patient (10 mg. per day) is within the range found satisfactory by Kupperman *et al.* (9).

Metabolic balance data

The metabolic balance data provided useful information in the management of this patient. We were impressed with the marked suppressive effect of 10 mg. of prednisone per day and the lack of alteration of the nitrogen, calcium, phosphorus, sodium, chloride and potassium balances at this dosage level. A dosage of 20 mg. of prednisone per day, however, resulted in a moderately negative nitrogen balance, a slight but definite increase in phosphorus excretion, initial sodium and chloride diuresis, and slightly negative potassium balance. Although our patient was well except for her adrenal abnormality, it is of interest to compare our balance data with those reported in subjects receiving cortisone or cortisone-like drugs. Nabarro *et al.* (40) reported the results of a 33-day metabolic balance study in a normal young adult male given hydrocortisone as the free alcohol (50 mg. daily for five days), 9-α-fluorohydrocortisone (3 mg. daily for five days), prednisone (30 mg. daily for five days), and cortisone acetate (150 mg. daily for three days). Little sodium retention was demonstrated during prednisone administration as compared to excessive retention with 9-α-fluorohydrocortisone, and moderate retention with hydrocortisone and cortisone. An initial potassium diuresis was seen on the first day of administration of prednisone, but it was transient and there was potassium equilibrium during the five-day period of prednisone administration. Negative nitrogen balance was observed while the patient received prednisone, exceeding that observed during cortisone and hydrocortisone therapy. Pechet and Bartter (41) carried out studies in Addisonian patients, comparing the effects of prednisone with those of cortisone and hydrocortisone. Slight sodium loss was observed during dosage with 30 mg. of prednisone

per day as compared to marked sodium retention during treatment with cortisone, 150 mg. daily. Transient potassium diuresis was also observed when prednisone was administered. A dosage of 30 mg. of prednisone per day produced nitrogen loss. Bunim *et al.* (42) found no retention of sodium and no loss of potassium or nitrogen when 30 mg. of prednisone was given daily for twelve days to 2 patients with rheumatoid arthritis. One patient receiving 50 mg. per day for twenty-four days manifested no change for ten days; then a slightly negative nitrogen balance developed. The small but definitely positive nitrogen balance observed during the control period in our study is of interest. It may be postulated that this was due to the excess of androgen and that the initial negative balance observed following administration of prednisone could be considered "beneficial" rather than undesirable, in that perhaps it was an index of eliminating the excess androgen and replacing it with less strongly anabolic estrogen.

The marked excretion of electrolytes and other substances in our patient on the fourth day of therapy with 20 mg. of prednisone per day warrants an attempt at explanation. Laidlaw and associates (43) observed that cortisone and hydrocortisone may produce an increase rather than a decrease in sodium excretion, particularly during the early period of hormone administration. It has been postulated (44) that hydrocortisone may either increase or decrease the excretion of sodium, because small doses may increase tubular reabsorption of sodium whereas large doses may increase sodium excretion due to the superimposition of a greatly increased glomerular filtration rate. Urinary creatinine excretion on the fourth day after administration of prednisone in our patient was approximately twice the daily value observed on several days preceding and on the day following that time. It appears, then, that increased glomerular filtration on the fourth day could explain the excessive excretion.

SUMMARY

The effect of prednisone therapy on a 29-year-old female with virilizing adrenal hyperplasia was studied for eighty days under metabolic balance conditions. A dosage of 20 mg. of prednisone per day resulted in effective suppression of urinary 17-ketosteroid excretion, but caused a moderately negative nitrogen balance, slightly increased phosphorus excretion, initial moderate sodium and chloride diuresis, and a slightly negative potassium balance. With a dosage of 10 mg. per day, effective suppression of 17-ketosteroid excretion continued but was not associated with any undesirable metabolic imbalances. Long-term prednisone therapy with a daily dose of 10 mg. maintained effective suppression of the excessive virilizing activity of this patient's adrenal glands without objectionable side-effects.

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RENAL FUNCTION STUDIES AND AUTOPSY REPORT IN A PATIENT WITH GIGANTISM AND ACROMEGALY*†

HERBERT GERSHBERG, M.D., HENRY O. HEINEMANN, M.D. AND HARRY H. STUMPF, M.D.‡

The Third Medical Division (New York University), Bellevue Hospital; and the Departments of Medicine, Physiology and Pathology, New York University College of Medicine, New York, N. Y.

IT IS generally accepted that hypersecretion of anterior pituitary growth hormone is the major factor in the production of gigantism and acromegaly. This assumption is supported by the fact that many of the abnormalities found in these patients can be reproduced in animals by injections of the hormone. These abnormalities include diabetes mellitus (1), arthritis (2), elevated level of plasma inorganic phosphate (3, 4), and increased body water (5, 6). In addition, a renotropic effect of the hormone has been demonstrated in dogs, in that glomerular filtration rate, renal blood flow, Tm_{PAH} (7), urea clearance and sulphate Tm (8) are increased. Glomerular filtration rate and renal plasma flow have been reported to be increased in acromegaly (9, 10), but to our knowledge there are no reports in which all the foregoing renal functions have been measured and correlated with observations at autopsy. This has prompted us to describe a case in which these measurements were made and in which autopsy revealed marked renal hypertrophy.

METHODS

For measurement of glomerular filtration rate and effective renal plasma flow a solution of inulin and *p*-aminohippurate (PAH) was infused intravenously with a constant-rate infusion pump. After at least three clearance periods, additional PAH was infused and the maximum tubular rate of transport (Tm_{PAH}) was determined for three more periods. Glucose Tm (Tm_G) was measured throughout the experiment, using arterial blood glucose levels. Though no exogenous glucose was infused, the load/T ratio remained between 1.2 and 1.3. After from three to five control Tm_G determinations,

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‡ Present address: The Mountainside Hospital, Montclair, N. J.

insulin was injected intravenously and Tm_G measured for the following forty-five minutes. Urea clearance was determined in five or six periods at urine flows above 2 ml. per minute. In the experiment performed on June 16, Na_2SO_4 was added to the infusion mixture to make a 5 per cent solution, for the simultaneous determination of Tm_{SO_4} at load/T ratios of 1.5 to 2.0.

Inulin was determined, after yeasting, by the method of Schreiner (11); blood and urine glucose by the Nelson-Somogyi method (12); PAH by the method of Smith *et al.* (13); urea by the method of Seligson and Seligson (14); inorganic phosphate by the method of Fiske and SubbaRow (15) and inorganic sulphate by the method of Power and Wakefield (16).

CASE REPORT

L. C., a 36-year-old colored man, was admitted to Bellevue Hospital on June 5, 1954 with fractures of both tibial plateaus incurred when he fell on arising from bed. About four months before, polyuria and polydipsia had developed and he had been told that he had diabetes mellitus. During this period he became progressively weaker and lost 40 pounds despite injections of 40 units of protamine zinc insulin daily.

The past history revealed that at the age of 12 he had begun to grow rapidly. At 23 he was hospitalized for meningitis and at this time an enlarged sella turcica was noted on x-ray examination. For the next four years he was on active duty with the Army. At 27, asthma developed, and since then he had given himself injections of epinephrine whenever necessary. He had never had headaches or blurred vision. He stated that his libido was normal.

On examination he was approximately 6 feet 6 inches in height, and weighed about 230 pounds (surface area, ± 2.37 sq.M.). His face was acromegalic in appearance, his body, hands and feet were large, and his chest was deep and wide. The blood pressure was 115/70 mm.Hg. Rhonchi and wheezes were heard in both lungs. The liver was not palpable, and there was no peripheral edema.

Analysis of the urine revealed 4^+ sugar and acetone. Analysis of the blood showed the following concentrations: blood glucose 400 mg. per 100 ml.; plasma sodium 140 mEq., chloride 110 mEq., and potassium 3.3 mEq. per liter; serum total protein 6.5 Gm. per cent, albumin 3.4 Gm. per cent, and cholesterol 302 mg. per 100 ml. The plasma level of urea varied from 23 to 33 mg. per 100 ml., and that of inorganic phosphorus from 2.1 to 3.5 mg. per 100 ml. The alkaline phosphatase level was 5.9 units. The white blood cell count was 7,500 with 72 per cent polymorphs, 10 per cent lymphocytes and 12 per cent eosinophils.

The patient continued to have glycosuria and acetonuria during treatment with 180 units of insulin daily. On June 16 (at this time acetonuria was 2^+) the filtration rate, urea clearance, Tm_G, Tm_{SO_4} and Tm_{PAH} were measured (Table 1). When corrected for surface area, the values were 60 to 100 per cent above normal. The renal plasma flow calculated from the clearance of PAH was only 641 ml. per minute. This low value is probably due to inadequate extraction of PAH as a result of the hyperglycemia. The blood glucose concentration at that time was 380 mg. per 100 ml., and at this level marked depression of C_{PAH} has been reported (17, 18).

The dosage of insulin was increased to 270 units of regular insulin daily, and though the glycosuria persisted, the acetonuria subsided. On June 24, determinations of glomerular filtration rate, renal plasma flow and glucose Tm were repeated, with essentially the same results. At this time the blood glucose concentration was 346 mg. per 100 ml. and the C_{PAH} was 691 ml. per minute.

TABLE 1. RENAL FUNCTIONS IN AN ACROMEGALIC MAN COMPARED WITH A NORMAL MAN*

	C_{In}		C_{Urea}		Tm_{SO_4}		$Tm_{Glucose}$		Tm_{PAH}	
	ml./min.	per Gm. KW†	ml./min.	per Gm. KW	μM./min.	per Gm. KW	mg./min.	per Gm. KW	mg./min.	per Gm. KW
Patient L.C.	325	—	159	—	289	—	10.68	—	165	—
Patient L.C. (values corrected to 1.73 sq. M.)‡	237	0.28	116	0.14	211	—	780	0.93	120	0.14
Normal man (per 1.73 sq. M.)§	131	0.44	70	0.23	40–100‖	—	375	1.25	76	0.25

* Average values during 3 to 5 clearance periods.
† Kidney weight.
‡ Calculated with estimated surface area of 2.37 sq. M.
§ Assumed mean normal kidney weight of 300 Gm. per 1.73 sq. M.
‖ Values from unpublished observations of Becker, Heinemann, Hodler and Gershberg (32).

To compare the effects of insulin on peripheral utilization of glucose and on tubular reabsorption of glucose, insulin was injected intravenously and the blood glucose concentration and Tm_G measured for the following thirty-two to forty-six minutes (Table 2). On June 16, 28 units (approximately 0.3 unit per Kg.) reduced the blood glucose level only 6 per cent in thirty-nine minutes while the Tm_G decreased 14 per cent. On June 24, after 56 units of insulin, the blood glucose concentration fell 10 per cent in twenty-nine minutes and the glucose Tm decreased 8 per cent.

During the following weeks, the insulin dosage was increased to 460 units daily but

TABLE 2. EFFECT OF INTRAVENOUS INSULIN ON BLOOD GLUCOSE LEVEL AND GLUCOSE TM IN A CASE OF GIGANTISM WITH ACROMEGALY

Exp. No.*	Time after insulin† (min.)	Arterial blood glucose (mg./100 ml.)	Glucose Tm (mg./min. per 100 ml. glom. filtr.)	Urine flow (ml./min.)	Plasma inorg. PO_4 (mg./100 ml.)
1	0	379	321, 325, 320‡	5.5	3.51
	10 (0–11)	374	315	4.9	—
	20 (11–22)	365	309	—	—
	28	361	—	4.9	—
	39 (22–32)	358	277	4.5	3.51
2	0	346	294, 277, 306‡	3.6	—
	13 (8–24)	326	280	3.5	—
	29 (24–37)	311	277	3.1	—
	41 (37–46)	311	288	2.9	—

* In experiment No. 1 (June 16), a dose of 28 units of regular insulin was injected intravenously; in experiment No. 2 (June 24) the dose was 56 units.

† The first number in each line refers to the time of blood collection, and the numbers in parentheses to the periods of urine collection.

‡ Values for three preceding control periods.

the glycosuria persisted. In one 24-hour period in which it was measured quantitatively, the glucose excretion was 194 grams. The daily urine volumes varied from 4,000 to 9,000 ml., and on one occasion reached 11,000 ml. It was extremely difficult to keep the patient on a C-350 diet because of extreme polyphagia. Nevertheless he remained in a comfortable state, though bedridden, until July 20 when he suddenly complained of a sharp pain in the left thigh and groin accompanied by generalized weakness. He was found to be sweating profusely and his pulse and blood pressure were unobtainable. Glucose and norepinephrine were injected intravenously but he died within a few minutes.

Autopsy report. There was an eosinophilic adenoma of the hypophysis approximately 3 cm. in diameter, with a mass measuring 1.0×0.5 cm. extending upward and anterior to the sella. There was also marked visceromegaly, which involved the heart (900 Gm.), kidneys (600 and 550 Gm.), lungs (1,600 and 1,500 Gm.), liver (7,500 Gm.), spleen (900 Gm.), and pancreas (25 cm. in length). The thyroid, adrenal and brain appeared proportional to the size of the body. The prostate and testes were small and the thymus was not identified.

The most striking changes were observed in the kidneys. The thickness of the cortex was 1.2 cm. There was marked enlargement of the glomeruli and tubules (Figs. 1 and 2). Dr. Jean R. Oliver of the Renal Research Unit, Overlook Hospital, Summit, New

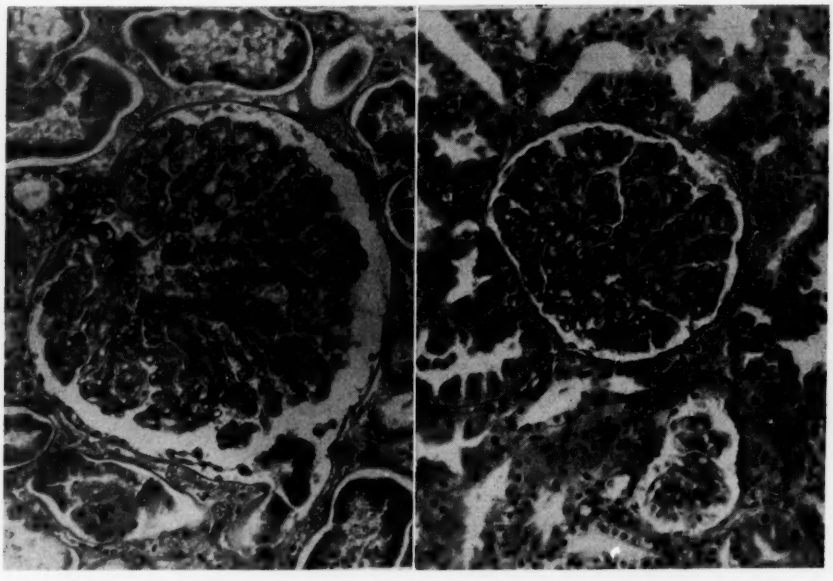

FIGURE 1 FIGURE 2

FIGS. 1 and 2. Representative glomeruli and convoluted tubules from kidney of acromegalic male (Fig. 1) and from normal kidney of male of same age who died in an automobile accident (Fig. 2). Note striking increase of glomerular size in Figure 1 as compared with normal glomerulus in Figure 2. The difference in tubular diameters is not as great. In the kidney from the acromegalic patient (Fig. 1) the capillary basement membrane is slightly increased in prominence and the capillary loops are congested. (Both photomicrographs, H. and E., 190×.)

Jersey made approximate measurements of the diameter of the glomeruli and proximal tubules. The average diameter of the glomeruli was 333 micra (normal, 212 micra) and of the proximal convoluted tubules 85 micra (normal, 65 micra). There was moderate deposition of glycogen in the tubular epithelium.

The heart was symmetrically enlarged (Fig. 3). The valve circumferences were as follows: tricuspid 15 cm., pulmonic 9 cm., mitral 15 cm. and aortic 7.5 cm. The thickness of the left ventricular wall was 2 cm. and of the right 0.5 cm. Moderate hypertrophy of the myocardial fibers was evident histologically. There was mild atherosclerosis of the coronary vessels, aorta and peripheral vessels.

FIG. 3. Heart (900 Gm.) of acromegalic patient on left compared with comparatively normal heart (360 Gm.) on right. Both hearts partially dissected. Note that the enlarged heart is, nevertheless, normal in shape.

Other important histologic observations were 1) thickening of the basement membrane of the seminiferous tubules, with reduced spermatogenesis and marked increase in interstitial connective tissue, 2) hypercellular bone marrow with an increase of the erythroid elements, 3) increased glycogen deposition in the liver cells, and 4) acute congestion of the lungs, spleen and kidney. The pancreatic acini and islets, and the thyroid and parathyroids were unremarkable.

The cause of death was not discovered. No pulmonary or cerebral embolus was found, and there was no evidence of a sudden increase in intracranial pressure.

COMMENT

Though the question is by no means settled, experimental evidence suggests that the growth hormone is the diabetogenic principle of the pitui-

tary[1] (19). Clinically, the association of diabetes mellitus with acromegaly is well known and the reported incidence varies between 10 and 40 per cent (22, 23).

In addition to the severe diabetes, Patient L. C. had marked visceromegaly. In particular, the lungs and liver were the heaviest, and the kidneys approximately the heaviest on record (24). The extraordinary size of the liver recalls the findings in canine acromegaly (25) and in rats treated with growth hormone (26), in which the ratio of liver weight to body weight was greater than the ratio for any other organ. The hepatomegaly in Patient L. C., however, probably reflects not only excess growth hormone activity, but also the metabolic derangements associated with prolonged uncontrolled diabetes.

It is not known whether the splanchnomegaly of acromegaly is a primary effect of the hormone, or is secondary to increased blood supply resulting from increased cardiac output. To our knowledge, cardiac output has not been measured in animals during treatment with growth hormone, or in acromegalics during the course of the disease.[2] Moreover, it is not known whether the cardiac hypertrophy is due to a direct effect of this hormone on cardiac musculature, or is secondary to increased circulatory demands. A direct effect on cardiac muscle is suggested by the marked increase in cardiac glycogen that occurs in rats twelve hours after a single injection of growth hormone (27). The rapidity with which the renal plasma flow and extracellular fluid volume increase in treated animals (8, 28) also suggests a direct effect on the circulatory system.

The increased glomerular filtration rate and Tm for PAH, glucose and SO_4 in Patient L. C. reflect the increased size of the glomeruli and proximal convoluted tubules seen on histologic examination. However, when the values are calculated per gram of kidney weight, it is found that the in-

[1] Raben and Westermeyer's suggestion (20) that the diabetogenic and growth-promoting factors of the pituitary are separate has been challenged on two grounds: that the doses necessary for these biologic actions differ, and that the pH of the injected extract has altered its physiologic action (21). When the Raben preparation was tested for its effect on protein anabolism (an integral part of growth) in dogs, it was found to be less active in reducing plasma urea concentration and urea excretion, and in inducing diabetes (8).

[2] Because of cyclic variations in growth hormone activity in acromegaly, isolated measurements in individual acromegalics do not indicate which change occurs first. Through the courtesy of Dr. Alfred P. Fishman, the cardiac output was measured in a 48-year-old acromegalic man (surface area of 1.8 sq.M.) who had had the disease for at least fourteen years. The cardiac output was normal (6 liters per minute) and his heart was not enlarged on x-ray examination. At that time his glomerular filtration rate was 178 ml./min./1.73 sq.M. and his renal plasma flow on two occasions was 653 ml./min./1.73 sq.M. and 844 ml./min./1.73 sq.M. This patient also had severe diabetes.

crease in function is not proportional to the organ weight (Table 1). Corcoran (personal communication) has found a normal $T^c_{H_2O}$ of 6.3 ml. per minute in an acromegalic, with a filtration rate of 320 ml. per minute and renal plasma flow of 1063 ml. per minute. This suggests that the hypertrophy is limited to the proximal tubules, since $T^c_{H_2O}$ is a distal tubular function (29), in contrast to Tm_{SO_4}, Tm_{PAH} and Tm_G which are functions of the proximal tubule.

In normal subjects and in patients with diabetes mellitus, administration of insulin reduces glucose Tm acutely by about 10 per cent (30, 31). In view of the slight effect of insulin on glucose Tm, it is difficult to draw conclusions from the results in our patient. There was a decrease in Tm_G ranging from 8 to 14 per cent, in contrast with the sluggish response of the blood glucose level. Thus it would appear that tubular sensitivity to insulin persists in the presence of resistance to insulin in other peripheral areas.

It is of interest that the plasma inorganic phosphate level was normal despite the widespread evidences of growth hormone activity. This may have been a result of phosphate loss in prolonged, uncontrolled diabetes.

SUMMARY

A study of a patient with gigantism, acromegaly and insulin-resistant diabetes is presented. The renal glomerular filtration rate and proximal tubular activities (Tm_G, Tm_{SO_4} and Tm_{PAH}) were markedly increased, reflecting the increase in size of the glomeruli and proximal tubules found at autopsy. At autopsy, splanchnomegaly was pronounced and the lungs, liver and kidneys were the heaviest on record.

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PARATHYROID DAMAGE IN MAN: MECHANISM OF EFFECT ON SERUM LEVELS OF CALCIUM AND PHOSPHORUS

STEPHEN M. KRANE, M.D.

The Medical Service of the Massachusetts General Hospital and the Department of Medicine, Harvard Medical School, Boston, Massachusetts

THE fall in the concentration of calcium in the serum of rats after parathyroidectomy has recently been shown to precede the expected rise in the concentration of phosphorus (1). This observation of Munson's confirms Greenwald's earlier findings (2) in dogs, and supports the concept that parathyroid hormone acts directly to maintain the level of calcium in the serum.

The chance observation of a patient who had marked hypocalcemia but a normal level of serum phosphorus at the time of the first sign of tetany following thyroidectomy for hyperthyroidism led to the search for similar patients to confirm, in man, the foregoing findings in animals.

METHODS

For the previous ten-year period, a review was made of the records of patients in whom permanent or transient symptoms of hypocalcemia had developed following thyroidectomy for hyperthyroidism, nodular goiter or carcinoma. Ten patients were selected who fulfilled the following criteria: 1) symptoms had developed within one to four days after operation, 2) a blood sample had been drawn at the first sign of tetany before any treatment was given, and 3) the blood sample had been analyzed for both calcium and phosphorus. None of these subjects had had symptoms suggestive of hypocalcemia before thyroidectomy, although preoperative calcium determinations had not been obtained. At operation, normal appearing parathyroids had been identified in all 10 patients. In 4, histologically normal parathyroid tissue had been identified in the surgical specimen; in the remaining 6, no parathyroid tissue had been found in the surgical specimen.

All chemical determinations were performed in the hospital chemistry laboratory. Calcium was determined by the methods of Fiske and Logan (3) and Elliot (4) (normal range 8.5–10.5 mg. per 100 ml.); and phosphorus by the method of Fiske and SubbaRow (5) (normal range 3.0–4.0 mg. per 100 ml.).

RESULTS

The data are summarized in Table 1. Thyroidectomy was for toxic diffuse goiter in 6 patients, carcinoma of the thyroid in 3, and nontoxic

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TABLE 1. SERUM CALCIUM AND PHOSPHORUS LEVELS AT THE TIME OF THE FIRST SIGN OF HYPOCALCEMIA AFTER THYROIDECTOMY

Patient	Sex and age	Postoperative diagnosis	Onset of hypocalcemic symptoms (postop. day)	Serum levels (mg./100 ml.) at time of onset of symptoms		Duration of hypoparathyroidism
				Ca	P	
F.W. 519840	M 18	Toxic diffuse goiter	3	4.9	4.0	Permanent
M.M. 624607	F 39	Adenocarcinoma	4	7.6	3.9	Permanent
E.L. 365865	F 24	Papillary carcinoma	4	6.5	3.9	Permanent
T.B.G. 745838	F 36	Toxic diffuse goiter	1	7.0	5.7	Permanent
J.B. 877372	F 30	Toxic diffuse goiter	1	6.3	4.1	Permanent
R.M. 775886	M 37	Toxic diffuse goiter	1	6.2	3.2	Transient
A.T. 462812	F 33	Papillary adenocarcinoma	3	7.6	3.5	Transient
A.S. 596085	F 36	Toxic diffuse goiter	2	6.1*	4.6	Permanent
K.G. 436563	F 33	Toxic diffuse goiter	3	5.3†	4.7	Permanent
E.R. 845885	F 42	Nontoxic nodular goiter	2	7.4‡	3.5	Transient

* Blood taken next morning after Chvostek sign present previous evening.

† ? Blood taken before treatment.

‡ No clinical sign of hypocalcemia until 3 days later.

nodular goiter in 1. In 6 of the 10 subjects the concentration of serum phosphorus was 4.0 mg. per 100 ml. or below when significant hypocalcemia was first recorded. Among the remaining 4 subjects, in only 1 was the serum phosphorus concentration above 4.7 mg. per 100 ml. at this time. All except 1 patient subsequently received some form of therapy for hypocal-

cemia, including calcium preparations, vitamin D, dihydrotachysterol, and aluminium phosphate gel. The natural history of the chemical changes in the blood could therefore not be observed. However, a delayed elevation in the level of serum phosphorus was observed in all subjects within a few days.

The initial blood sample was drawn after an overnight fast, or several hours after eating, obviating the possible hypophosphatemic effect of carbohydrate ingestion. Seven subjects were permanently hypoparathyroid; one of them (M. M.) died in 1954 from metastatic adenocarcinoma of thyroid origin.

DISCUSSION

These observations indicate that damage to, or removal of, parathyroid tissue incidental to thyroid surgery results first in hypocalcemia despite a normal concentration of phosphorus in the serum. The elevation in phosphorus concentration is a delayed event. This is contrary to the hypothesis (6) that the parathyroids act to maintain the level of calcium in the serum only indirectly by regulation of the concentration of inorganic phosphate in the serum through control of the renal excretion of phosphate. If the latter were true, then the serum phosphorus level would already be elevated by the time the calcium level was depressed. The results in man are in agreement with those obtained by Munson and by Greenwald in animals.

Albright (6) noted hypocalcemia without hyperphosphatemia upon removal of hyperplastic or adenomatous parathyroids in cases of osteitis fibrosa. He postulated a "sucking" into the skeleton of both calcium and phosphorus in the process of repair of the osteitis. In the present series this hypothesis could explain the findings in the thyrotoxic patients who may have had a skeletal disorder, functionally (7) and pathologically (8) that of osteitis fibrosa. However, it seems unlikely that this mechanism could account for the identical results in the patients with thyroid carcinoma. Alkaline phosphatase determinations were recorded in only 3 patients and were significantly elevated only in the patient (T. B. G.) who had definite hyperphosphatemia initially.

CONCLUSIONS

Parathyroid damage incidental to thyroidectomy in 7 of 10 patients resulted in a fall in the level of serum calcium associated with little or no increase in the level of inorganic phosphate. This supports the concept that the parathyroids act directly to maintain the level of calcium in the serum.

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RESPONSE OF PLASMA 17,21-DIHYDROXY-20-KETOSTEROIDS TO REPOSITORY CORTICOTROPIN

J. GELLER, M.D.,* J. L. GABRILOVE, M.D.
AND L. J. SOFFER, M.D.

The Endocrine Research Laboratory and Clinic of the Department of Medicine, and the Department of Chemistry, The Mount Sinai Hospital, New York, N. Y.

INTRODUCTION

THE widespread use of long-term therapy with corticotropin (ACTH) in the treatment of numerous medical disorders has stimulated the search for a potent and long-acting intramuscular repository form. The introduction of corticotropin in gelatin (Gel-ACTH) in 1952 and more recently corticotropin with zinc hydroxide (Zn-ACTH) have made available two such preparations for clinical use. The sustained effectiveness of a single intramuscular dose of these preparations has been measured in the past primarily by indirect laboratory tests of increased function of the adrenal cortex and by the clinical responsiveness to therapy. Levin (1) reported clinical improvement for twelve to twenty-four hours following one injection of 40 to 60 units of Gel-ACTH. Thorn (2) reported a depression in the number of circulating eosinophils and an increase in the urinary excretion of neutral 17-ketosteroids for twelve to twenty-four hours following a single injection of the same repository preparation. Zn-ACTH was studied by Greene (3), den Oudsten (4), and Turner (5) who reported a depression in the number of circulating eosinophils, an increase in the urinary excretion of neutral 17-ketosteroids and clinical improvement for a period of twenty-four to forty-eight hours following a single injection. More recently, Bayliss (6) studied the adrenocortical response to the administration of Gel-ACTH by measuring the plasma 17-hydroxycorticoids. He found that 80 units of this preparation given intramuscularly caused an elevation of the 17-hydroxycorticoid plasma levels for four to eight hours, whereas 20 units given by the same route resulted in a peak response at two to four hours.

Because of the difficulty in obtaining quantitative data by means of indirect laboratory and clinical criteria, it was felt that the measurement of the plasma 17,21-dihydroxy-20-ketosteroids afforded a more direct and

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* Trainee, National Institute of Arthritis and Metabolic Diseases.

objective measure of adrenocortical function, particularly as a basis for comparing the response to various repository corticotropin preparations. The purpose of this study was to compare the potency of repository Zn-ACTH and Gel-ACTH by means of their effect on the level of the plasma 17-hydroxycorticosteroids at various intervals following single intramuscular injections of each in equal dosage.

METHODS AND MATERIAL

These studies were conducted on hospitalized patients without evidence of endocrine dysfunction. No patient had been treated with adrenocortical steroids or corticotropin for at least three months prior to study. No patient was studied who was acutely ill or had been exposed to recent stress. All patients were given the corticotropin between 9:00 and 10:00 A.M., after breakfast. No meals were withheld during the test period. The corticotropin preparations used were Cortrophin-Gel[1] and Cortrophin-Zinc,[1] and they were always administered intramuscularly by the same person. Blood samples (40 cc.) were drawn at 0, 2, 8 and 24 hours in all patients, and also at the end of 30 hours in 5 patients. The blood samples (heparinized) were centrifuged within one hour of collection and the plasma removed and frozen until assayed. All samples were analyzed within one week of collection.

Plasma 17,21-dihydroxy-20-ketosteroids (referred to as 17-hydroxycorticoids) were determined by the method of Silber and Porter (7). All determinations were made in duplicate.

RESULTS

Zn-ACTH

Twelve studies following the intramuscular administration of 40 units of Zn-ACTH were carried out in 11 patients. In all, a significant rise in the level of plasma 17-hydroxycorticoids (Table 1) to an average of 2.3 times the control level occurred at two hours. This rise either increased or remained approximately the same in 8 of the 10 patients in whom an eight-hour blood specimen was obtained; in the remaining 2, the concentration returned to control values. At twenty-four hours the level of plasma corticosteroids returned to control values in all except 2 cases; in 1 of these an increased titer was still found at thirty hours.

At two and eight hours the average rise was to a level 2.3 times that of the control value, the maximal increase varying from 1.8 to 4.5 times the initial titer. In 1 patient (H. S.), results of identical studies carried out two weeks apart showed good agreement. The response to the administration of 20 units of Zn-ACTH was tested in 3 patients and the pattern of response was similar to that obtained following the use of 40 units (Table 1).

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[1] Cortrophin-Gel, brand of purified corticotropin in gelatin, and Cortrophin-Zinc, brand of corticotropin with zinc hydroxide, furnished by Dr. K. W. Thompson, Organon Inc.

TABLE 1

Type of ACTH	Dose (units)	Patient		Level of plasma free 17-OH-CS (γ/100 ml.)					Maximal increase expressed as multiple of control value
				Hours after ACTH					
		Sex	Age	0	2	8	24	30	
Zn	40	M	58	13	40	48	37	8	3.7 [8]†
Zn	40	F	25	25	40	62	44	39	2.5 [8]
Zn	40	F	53	15	41	44	19	12	2.9 [8]
Zn	40	M	60	14	42	—	13	3	3.0 [2]
Zn	40	M*	45	13	31	32	14	—	2.5 [8]
Zn	40	M*	45	15	37	32	12	—	2.3 [2]
Zn	40	F	21	15	35	—	18	—	2.3 [2]
Zn	40	F	22	22	45	38	13	—	2.0 [2]
Zn	40	M	54	18	26	33	12	—	1.8 [8]
Zn	40	M	55	11	23	9	—	8	2.0 [2]
Zn	40	M	60	6	27	11	5	—	4.5 [2]
Zn	40	M	28	18	29	38	18	—	2.1 [8]
Zn	20	M	14	22	37	29	19	—	1.7 [2]
Zn	20	M	61	20	36	40	16	—	2.0 [8]
Zn	20	M	60	17	28	27	—	—	1.6 [2]
Gel	40	M	18	13	38	7	22	—	3.0 [2]
Gel	40	M	21	13	40	23	9	—	3.0 [2]
Gel	40	M	23	18	40	8	21	—	2.2 [2]
Gel	40	M	25	24	38	13	14	—	1.6 [2]
Gel	40	M	60	9	26	7	—	—	3.0 [2]
Gel	40	M	54	17	45	25	—	—	2.6 [2]

* Indicates the same patient.

† Figures in brackets indicate time (hours) of occurrence of maximal increase.

ACTH-Gel

In 6 patients who were given 40 units of Gel-ACTH, the pattern of response was uniform (Table 1). A peak response averaging 2.3 times the control values occurred at two hours, followed by a return to control levels by eight hours.

DISCUSSION

Determination of the plasma level of free 17-hydroxycorticosteroids affords the most direct measurement of adrenocortical secretory function available at present. The technique readily lends itself to an objective study of the duration of effectiveness of repository forms of corticotropin.

In this study the elevated levels of plasma 17-hydroxycorticoids present eight hours after intramuscular administration of Zn-ACTH were in rather marked contrast to the return to control levels found eight hours after injection of comparable amounts of Gel-ACTH. The maximal levels attained, however, were very similar following injection of equal amounts (40 units) of each preparation (Fig. 1). In the few patients receiving 20 units of Zn-ACTH, a peak response similar in pattern to that seen in those receiving 40 units was noted, although the maximum level attained was perhaps slightly less. The concept of maximal adrenal responsiveness has been

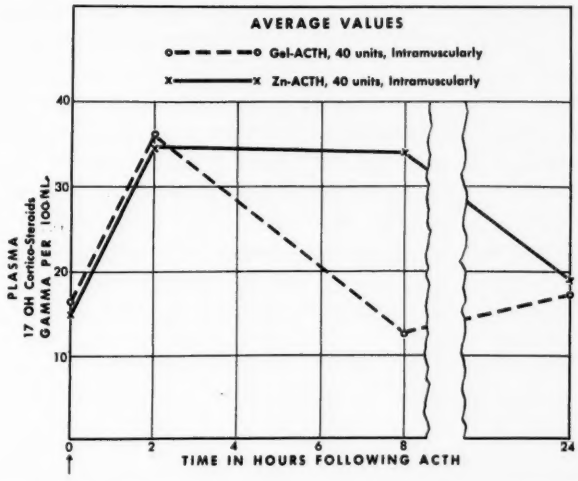

FIG. 1. Course (in 24 hours) of the plasma 17-OH-corticosteroid level following Gel-ACTH compared with that following Zn-ACTH

dealt with by several investigators. Eik-Nes (8) demonstrated maximal adrenal stimulation with 25 units of lyophilized corticotropin given intravenously over six hours; Bayliss (6) showed that 1 unit of aqueous corticotropin per hour administered intravenously afforded maximal adrenal stimulation. Thorn (2) has stated that the intravenous administration of 2 units of aqueous corticotropin per hour affords maximal adrenal secretion. The absolute values for plasma 17-hydroxycorticoid concentration observed by us eight hours following the intramuscular administration of Zn-ACTH are similar to the peak values reported by Bayliss (6) and Eik-Nes (8) during maximal stimulation with intravenously administered corticotropin given over an eight-hour period. The achievement of plasma steroid levels at least equal in duration and degree to those obtained following the intravenous administration of 20 to 25 units of corticotropin

over an eight-hour period is thus possible with a single intramuscular injection of Zn-ACTH.

SUMMARY AND CONCLUSIONS

1. The plasma levels of 17,21-dihydroxy-20-ketosteroids (17-OH-CS) were significantly elevated in all of 11 non-endocrine patients two hours following the intramuscular administration of 20 to 40 units of Zn-ACTH. In 8 of 10 patients in whom eight-hour determinations were made, similar levels were observed at the end of this period.

2. In 6 patients given 40 units of Gel-ACTH intramuscularly, a peak response of the plasma 17-OH-CS concentration was observed at the end of two hours, followed by a return to control levels in all instances at eight hours.

3. The results of this study do not imply that results of previous clinical studies employing measurements of urinary neutral 17-ketosteroids and circulating eosinophils are invalid in claiming a duration of twenty-four to forty-eight hours for the response to a single injection of repository corticotropin.

4. Standardization of long-acting preparations of corticotropin now appears possible by measuring plasma steroid levels at specified times following the administration of repository corticotropin to human subjects.

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THE METABOLISM OF 1-DEHYDRO-17-HYDROXY-CORTICOSTEROIDS IN HUMAN SUBJECTS

W. ROY SLAUNWHITE, JR., PH.D. AND
AVERY A. SANDBERG, M.D.*
Roswell Park Memorial Institute, Buffalo, N. Y.

EVEN though the 1-dehydro-17-hydroxycorticosteroids (Δ^1-17-OHCS) have been in clinical use for some time and have proved to be effective in much smaller doses than the 17-hydroxycorticosteroids (cortisone, cortisol), only one brief article (1) has appeared concerning their metabolism in human subjects. In this paper we wish to present studies on the metabolism of 1-dehydrocortisol (Δ^1-F) and 1-dehydrocortisone (Δ^1-E) in man, with particular emphasis on the differences observed when compared with the metabolism of cortisone and cortisol.[1] The metabolism of 4-C^{14}-1-dehydrocortisol will be the subject of a separate report.

METHODS

Patients of both sexes between the ages of 7 and 66 years were hospitalized on the Metabolic Ward and given steroids by oral ingestion (10–240 mg. per day) or by intravenous injection (1 mg. per Kg. body weight). The oral steroids were given in tablet form as prednisone (Meticorten) or prednisolone (Meticortelone). The intravenous steroids were injected either as the hemisuccinates dissolved in small amounts of an aqueous solution, or as the free alcohols in a 5 per cent ethanol solution. The levels of unconjugated 17-hydroxycorticosteroids[2] (17-OHCS) in the plasma were determined by the method of Nelson and Samuels (3). Urines were collected with preservative (toluene) and analyzed for 17-OHCS by the method of Glenn and Nelson (4) and for 17-ketosteroids (17-KS) by the method of Klendshoj *et al.* (5), using the Allen correction as demonstrated by Chang and Slaunwhite (6).

4-C^{14}-cortisol[3] was dissolved in 1 or 2 ml. of ethanol and diluted in 30 ml. of physiologic sodium chloride before being injected intravenously. Blood samples were collected and analyzed by methods described elsewhere (7). Small samples of urine were evaporated in steel planchets and the radioactivity was determined in a windowless pre-flush

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* Scholar in Cancer Research of the American Cancer Society (1953–56).

[1] A preliminary report (2) was presented at the meeting of the American Society of Biological Chemists, April 16–20, 1956, Atlantic City, N. J.

[2] The terms "Porter-Silber chromogen(s)" and "17-hydroxycorticosteroid(s)" are used almost interchangeably when referring to the method of assay, since the former is used as a measure of the latter.

[3] Obtained through the courtesy of the Endocrinology Study Section of the National Institutes of Health.

counter. The bulk of the urine was hydrolyzed either by boiling with 15 volumes per cent of hydrochloric acid for ten minutes or by the mild enzymatic method as described by Sandberg and Slaunwhite (7). The crude extracts were "purified" by chromatography on Florisil (7) and the radioactivity counted. The "purified" extracts of 17-KS were then subjected to gradient elution chromatography according to the method of Lakshmanan and Lieberman (8). The identity of individual peaks was checked by paper chromatography (9), sulfuric-acid chromogens (10), and infrared spectroscopy using the recently developed KBr technique (11).

The binding of Δ^1-F to plasma proteins was determined by an ultrafiltration technique to be described in detail elsewhere (12).

RESULTS

The rate of disappearance of intravenously injected Δ^1-F was essentially indistinguishable from that of cortisol (F) (Fig. 1), except for the one-hour point on the F curve of Patient J. B., which was low due to loss. However, the concentration of Δ^1-F was approximately 50 per cent higher than that shown, since the chromogenicity of Δ^1-F is two-thirds that of F in the Porter-Silber reaction. The data on the binding of Δ^1-F and F to plasma proteins are presented in Table 1. Approximately 60 per cent of both Δ^1-F and F was bound *in vitro* to normal plasma. The close agreement with the data on the binding capacity of fraction V (albumin) indicates that this fraction may account for most of the binding in plasma. The values reported for F are also in agreement with values found for 4-C^{14}-F, which will be reported elsewhere (12).

It is well known that the administration of large amounts of 17-OHCS induces modest increases in urinary 17-KS. In the present study, however, analysis of the urine revealed that not only was there no detectable conversion of Δ^1-17-OHCS to 17-KS, but administration of large amounts of Δ^1-17-OHCS inhibited endogenous production of 17-KS (Fig. 2, 3 and 4). Simultaneously, the urinary excretion of unconjugated Porter-Silber chromogens was greatly enhanced in the case of Δ^1-F, at the expense of the conjugated chromogens (Table 2, Fig. 5). Normally the unconjugated 17-OHCS do not constitute more than 5 to 10 per cent of the total determinable 17-

TABLE 1. *In vitro* BINDING TO HUMAN PLASMA PROTEINS

Steroid	Steroid conc'n. (μg./ml.)	Protein	Per cent bound	Per cent recovery
Δ^1-F	20	5 Gm. % fraction V	53	105
F	20	5 Gm. % fraction V	67	108
Δ^1-F	20	Plasma	64	100
F	20	Plasma	56	105

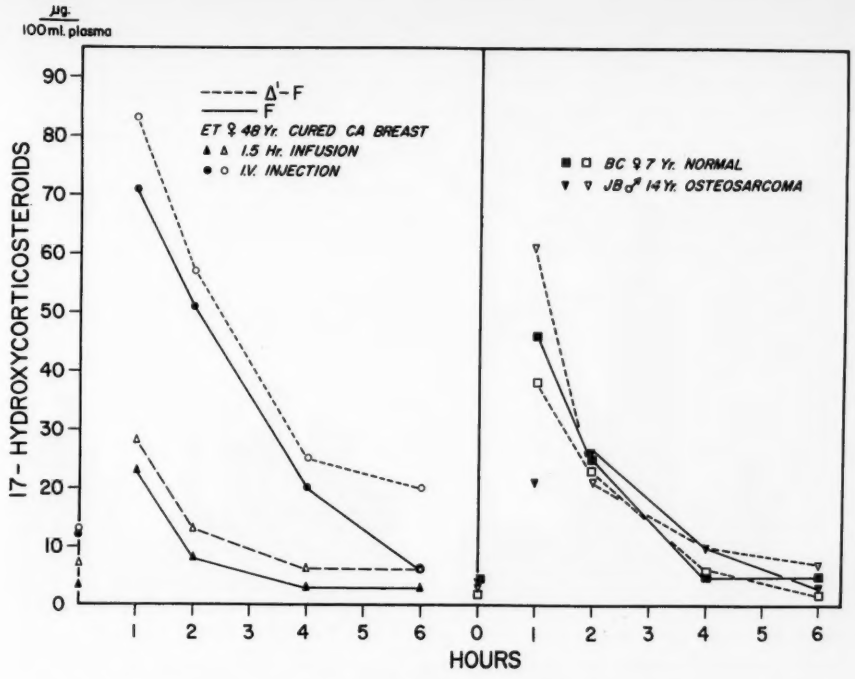

FIG. 1. Disappearance of unconjugated Porter-Silber chromogens from plasma after intravenous injection of steroid. Concentrations are based on cortisol as standard.

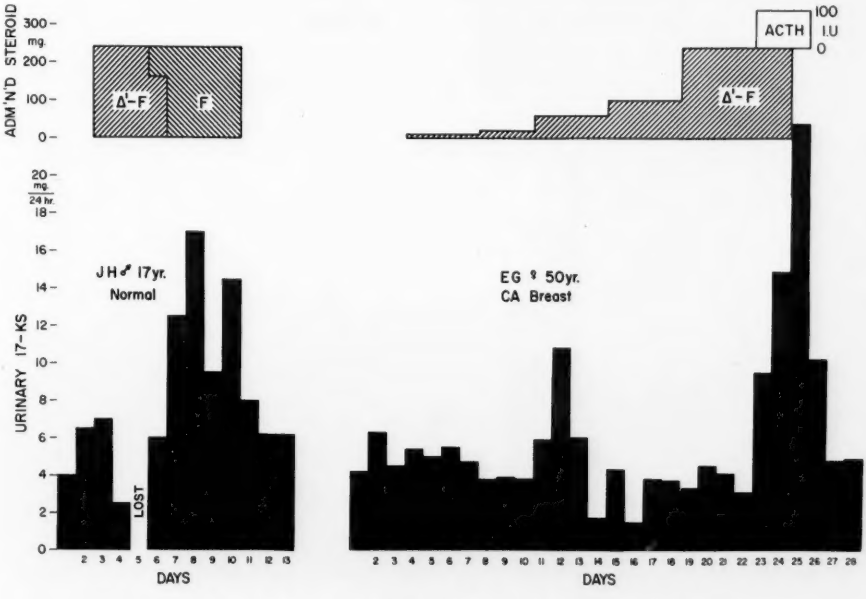

FIG. 2. Urinary excretion of 17-KS after oral ingestion of steroids and after intramuscular injection of ACTH.

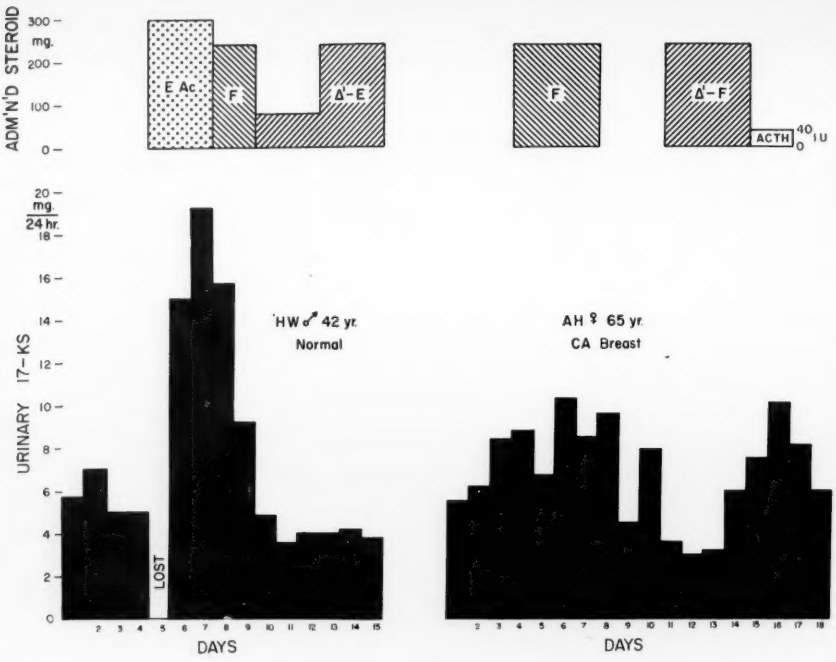

Fig. 3. Urinary excretion of 17-KS after oral ingestion of steroids and after intramuscular injection of ACTH.

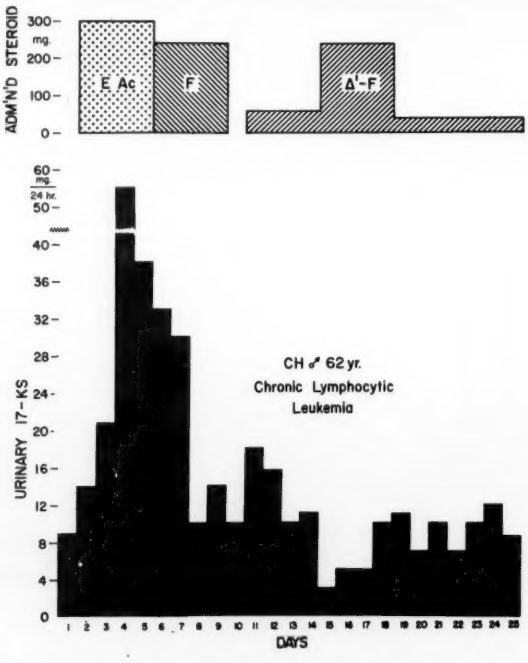

Fig. 4. Urinary excretion of 17-KS after oral ingestion of steroids.

TABLE 2. URINARY EXCRETION OF PORTER-SILBER CHROMOGENS (PSC)

Patient and Diagnosis	Sex and Age	Steroid administered	Urinary PSC (mg./24 hrs.)*			
			Free	Conj.	Total	Conj./Free
E.T.—Cured Ca of breast	F 48	70 mg. F (hs),† i.v.	1.0	9.2	10.2	9.2
		70 mg. Δ^1-F (hs), i.v.	10.9	4.4	15.3	0.4
		70 mg. F, i.v.	1.8	16.2	18.0	9.0
		70 mg. Δ^1-F, i.v.	10.6	6.7	17.3	0.6
B.C.—Normal	F 7	40 mg. F (hs), i.v.	0.8‡	9.9‡	10.7‡	12
		40 mg. Δ^1-F (hs), i.v.	3.0‡	2.8‡	5.8‡	0.9
J.B.—Osteosarcoma	M 14	45 mg. F (hs), i.v.	0.8	12.4	13.2	15
		45 mg. Δ^1-F (hs), i.v.	7.8	7.7	15.5	1.0
A.H.—Ca of breast	F 65	240 mg. F§	0.3	9.0	9.3	30
		240 mg. F§	3.3	6.2	9.5	1.9
		240 mg. Δ^1-F§	8.7	2.4	11.1	0.3
		240 mg. Δ^1-F§	19.9	7.8	27.7	0.4
		ACTH (100 I.U. gel)	4.6	12.9	17.5	2.8
		—	0.9	8.0	8.9	8.9
H.F.—Malignant melanoma	M 25	240 mg. F§	3.7	61.1	64.8	16
		—	1.4	24.1	25.5	18
		240 mg. Δ^1-F§	30.2	39.2	69.4	1.3
		—	0.1	0.8	0.9	8
		—	0.6	11.0	11.6	19
E.G.—Ca of breast	F 50	10 mg. Δ^1-F§	0.9	2.5	3.4	2.8
		20 mg. Δ^1-F§	2.2	2.0	4.2	0.9
		60 mg. Δ^1-F§	10.3	5.3	15.6	0.5
		100 mg. Δ^1-F§	17.0	4.8	21.8	0.3
		240 mg. Δ^1 F§	35.8	8.4	44.2	0.2
		240 mg. Δ^1 F§ ACTH	41.6	1.9	43.5	0.05
		ACTH (100 I.U. gel)	43.6	18.6	62.2	0.3

* Cortisone used as a standard.
† Hemisuccinate.
‡ Mg./Gm. creatinine.
§ Divided into 8 doses for the day.

OHCS, but after a single injection of Δ^1-F, 50 per cent of the urinary 17-OHCS remained unconjugated. Upon continual administration of Δ^1-F, as much as 95 per cent was unconjugated, even during administration of corticotropin.

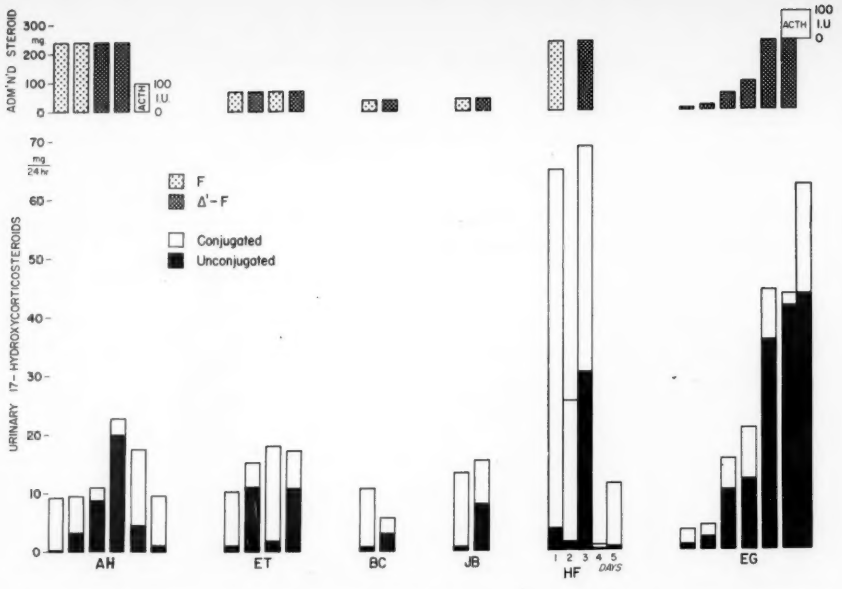

FIG. 5. Urinary excretion of unconjugated and conjugated Porter-Silber chromogens after administration of F, Δ^1-F and/or ACTH. The results in Patients A.H. and E.G. are on urine samples selected during prolonged therapy with the drugs and doses indicated. Patients E.T., B.C. and J.B. received single intravenous injections.

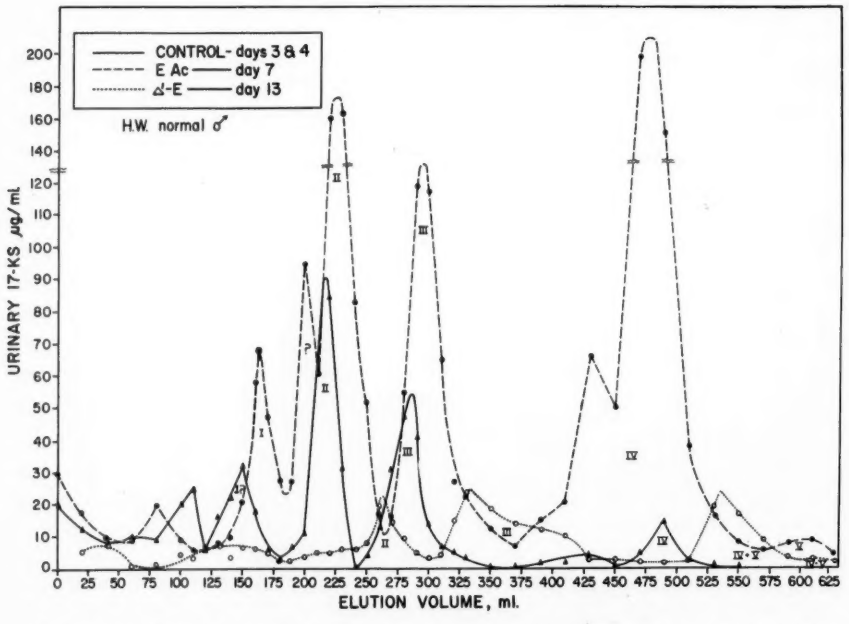

FIG. 6. Analysis of urinary 17-KS of Patient H.W. (*see* Fig. 3) by gradient elution chromatography. I, dehydroisoandrosterone; II, androsterone; III, etiocholanolone; IV, 11-ketoetiocholanolone; V, 11β-hydroxyetiocholanolone. Identities were confirmed by paper chromatography and infrared spectroscopy.

Examination of urinary 17-KS by gradient elution chromatography showed more striking differences than were evident by analysis of the crude extracts (Fig. 6). Compared to the control period, administration of Δ^1-E produced a marked decrease in the amount of most compounds, and dehydroisoandrosterone disappeared entirely. 11-Hydroxyetiocholanolone was present after Δ^1-E, but not in the control period.

That Δ^1-17-OHCS do not interfere with the conversion of F to 17-KS can be seen from Table 3. The conversion of 4-C^{14}-F to radioactive 17-KS

TABLE 3. URINARY EXCRETION OF RADIOACTIVE 17-KETOSTEROIDS AFTER INJECTION OF 2 μC. OF 4-C^{14}-CORTISOL AND STEROIDS DESCRIBED BELOW

Patient and Diagnosis	Sex and Age	Day	Steroid administered orally	Radioactivity of urinary 17-KS (counts/min.)
F.U.—Ca of prostate	M 66	1	240 mg. Δ^1-E	45,000
		2	—	12,000
J.H.—Normal	M 17	1	240 mg. Δ^1-F	lost
		2	—	10,000
		8	240 mg. F	83,000
		9	—	12,000
C.H.—Chronic lymphocytic leukemia	M 62	1	240 mg. Δ^1-E	33,000*
		2	—	41,000

* Injected at 5:30 P.M. instead of 9:30 A.M. as others were; all urines were collected from 7:00 A.M. to 7:00 A.M.

during the administration of Δ^1-E or Δ^1-F was not significantly different from the conversion observed during the administration of F; nor was there any change in the rate of disappearance of 4-C^{14}-F from the plasma (Fig. 7).

The results of paper chromatography of the fractions containing the unconjugated Porter-Silber chromogens were interesting, although not definitive in most cases due to lack of standards. The principal compound noted after administration of Δ^1-F was Δ^1-F itself, particularly when a dose of 45 or more milligrams was taken orally daily (Table 4). Like pure 1-dehydrocortisol, this compound absorbed light in the ultraviolet area at 240 mμ but failed to fluoresce when treated with 10 per cent NaOH (9), indicating that it was not a Δ^4-3-ketosteroid but probably a $\Delta^{1,4}$-3-ketosteroid or a Δ^1-3-ketosteroid. It reduced triphenyltetrazolium chloride (TPTZ) and failed to react with periodic acid (13), indicating a ketol rather than a glycerol side chain. This is consonant with its reactivity in the Porter-

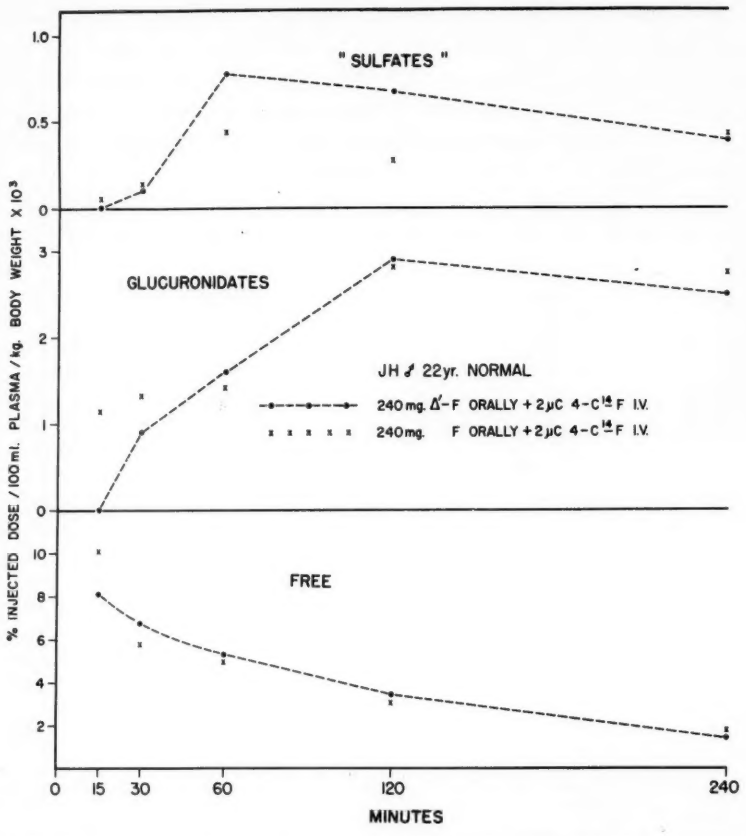

FIG. 7. Disappearance from the plasma of unconjugated radioactive substances and the appearance of radioactive substances liberated by β-glucuronidase and by continuous extraction at pH 1 after intravenous injection of 4-C^{14}-F during the prolonged oral administration of F or Δ^1-F.

Silber reaction which indicates a dihydroxy-acetone side chain. The sulfuric-acid chromogen test with pure Δ^1-E produced an absorption maximum at 263 mμ, a shoulder (plateau) at 340 mμ, and a broad maximum centered at 420 mμ; with Δ^1-F the maxima were at 267 and 359 mμ. The substances having an Rf of approximately 0.20 were identical with authentic Δ^1-F by this test. The infrared spectrum of the acetate was also identical with that of the standard. In addition, there were several other spots with Rf values both higher and lower than that of Δ^1-F. These spots absorbed in the ultraviolet region, did not fluoresce after treatment with NaOH (9), reduced TPTZ only occasionally, and did not react with per-

TABLE 4. PAPER CHROMATOGRAPHY OF URINARY 17-HYDROXYCORTICOSTEROIDS

Pat.	Steroid admin'd	State	Rf(u.v.)	TPTZ	Pat.	Steroid admin'd	State	Rf (u.v.)	TPTZ
E.G.	60 mg. Δ^1-F (oral)	Free	.008	+	E.T.	70 mg. Δ^1-F (i.v.)	Free	.018	0
			.050	0				.050	0
			.139	0				.196*	+
			.209*	+			Conj.	.021	+
			.605	0				.342	0
E.G.	240 mg. Δ^1-F (oral)	Free	.009	+				.413	0
			.053	0				.790	0
			.220*	+	J.B.	45 mg. Δ^1-F (i.v.)	Free	.015	+
J.B.	45 mg. Δ^1-F (i. v.)	Conj.	.029	+				.047	0
			.335†	0				.193*	+
					S.S.	240 mg. Δ^1-E (oral)	Free	.019‡	0
								.227*	+

* Identified as Δ^1-F.
† Yellow fluorescence with 10% NaOH.
‡ Part of the spot reacted positively with TPTZ and fluoresced with 10% NaOH.

iodic acid (13). Their identity remains unknown. Hydrolysis with β-glucuronidase liberated at least four substances, but none could be identified.

After administration of 240 mg. of Δ^1-E, the principal metabolite in the urine was again Δ^1-F. This is in agreement with the results reported by Vermeulen (1).

DISCUSSION

It is apparent from the data presented that the addition of a Δ^1 double bond to cortisone and cortisol has a profound effect upon their metabolism. They are not converted appreciably, if at all, to 17-KS even at dosage levels as high as 240 mg. daily. According to Dorfman and Ungar, the bulk of the 17-OHCS must be reduced in ring A before the side chain is removed (14). It is logical to suppose, then, that the additional double bond in ring A prevents attachment of the Δ^1-17-OHCS to the hydrogenase system involved in the reduction of the α,β-unsaturated ketone. This is not a competitive reaction, as shown by the fact that the administration of 240 mg. daily of Δ^1-17-OHCS did not influence the conversion of 2 micromoles of 4-C^{14}-cortisol to radioactive 17-KS. (A more detailed study of the 17-KS formed from 4-C^{14}-cortisol will be presented in another paper.) On the other hand, the presence of the Δ^1 double bond did not interfere with the reduction of the 11-ketone group, since when Δ^1-E was administered, most of it was excreted as free Δ^1-F. Similar reduction of the 11-ketone group has been observed during the administration of cortisone, with the consequent isolation of hydrocortisone from the urine (15, 16).

The most surprising aspect of the study was the large amount of the administered compound (Δ^1-E or Δ^1-F) which is excreted unconjugated. Again, although no definitive statement can be made, it is reasonable to assume that the Δ^1 double bond accounts for the abnormal behavior. Without reduction of the α,β-unsaturated ketone in ring A to an alcohol, conjugation does not occur.

In some ways, the metabolism of 1-dehydrocorticosteroids closely resembles that of 17-OHCS. Both are readily absorbed from the intestinal tract, since urinary excretion of their metabolites is as great after oral ingestion as after intravenous injection. Both are cleared from the plasma at approximately the same rate, and the presence of a large quantity of Δ^1-F does not affect significantly the rate of clearance of radioactive F. Both are bound to plasma proteins to about the same extent.

Why then are the Δ^1-17-OHCS equally as effective clinically as the 17-OHCS at approximately a fifth the dosage? It is not due to increased retention in the plasma, since the rate of clearance is essentially the same. It is not due to a slower absorption from the gastro-intestinal tract and hence a more prolonged action, since consistent results could be obtained only when the total dose was subdivided. The results in earlier patients receiving one dose daily were erratic and are not included in the present paper. It probably is not due to increased absorption by tissues, for then urinary excretion would be slower, and it is apparent from the data that the rate of urinary excretion of Δ^1-17-OHCS metabolites is at least as rapid as that of 17-OHCS. In Patient H. F., for example, the fall in urinary 17-OHCS the day after administration of Δ^1-F was more pronounced than after administration of F.

Even though the concentration of Porter-Silber chromogens in the plasma following Δ^1-17-OHCS administration is somewhat higher than that following F, we do not believe that these differences can account entirely for the greater clinical effectiveness of the Δ^1-steroids. It should be pointed out that the methods used for the estimation of blood and urinary Δ^1-17-OHCS and 17-OHCS and their metabolites determine only those steroids with a dihydroxy-acetone side chain. It is possible that other metabolites, not determinable by the procedures used and for which no good methods of estimation are available at present, play an important part in the understanding of the differences in the metabolism of these steroids. We hope that our experiments with radioactive Δ^1-F will shed more light on this aspect.

It has been demonstrated that cortisol is clinically effective, whereas its reduced forms, dihydrocortisol and tetrahydrocortisol, are not. Since the latter two compounds are Porter-Silber chromogens, it is likely that the concentration of *effective* steroid decreases more rapidly in the case of F

than in the case of Δ^1-F. This hypothesis is supported by the fact that a great part of Δ^1-F is excreted in the urine unreduced.

The other possibility is that Δ^1-F is simply more active at a molecular level, due to a slightly altered structure and perhaps a better fit with the enzymes involved.

Δ^1-17-OHCS apparently exert their influence upon adrenal activity indirectly by way of the pituitary, since concomitant injection of corticotropin produced the expected results—increased urinary excretion of 17-OHCS and 17-KS.

SUMMARY

The metabolism of 1-dehydrocortisol and of 1-dehydrocortisone in human subjects was compared to that of cortisol and cortisone. In contrast to 17-hydroxycorticosteroids (17-OHCS), which are converted appreciably to 17-ketosteroids (17-KS), the Δ^1-17-OHCS are not significantly converted to 17-KS and actually suppress urinary 17-KS excretion. The determinable Δ^1-17-OHCS excreted in the urine are largely unconjugated and for the most part unchanged. The clinical effectiveness of the 1-dehydro compounds is postulated to be due, at least in part, to the body's inability to metabolize the Δ^1-17-OHCS in the same manner as cortisol and cortisone. The latter are rapidly reduced to the physiologically inactive dihydro and tetrahydro compounds, whereas the 1-dehydro compounds are excreted to a great extent unmetabolized. The administration of Δ^1-17-OHCS does not interfere with the normal metabolism of 4-C^{14}-cortisol. The suppression of adrenal function is mediated through the pituitary.

Acknowledgments

We are indebted to Dr. Kristen Eik-Nes of the University of Utah for the analysis of plasma 17-hydroxycorticosteroids; to Miss Lucille Pucko, Mr. Lawrence Beecher and Mr. Eugene Barry for technical assistance; and to Dr. C. J. O'Donovan of the Upjohn Company and to Dr. Frederick K. Heath of Merck and Company for the 1-dehydrocortisol and 1-dehydrocortisone used in the studies.

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URINARY PORTER-SILBER CHROMOGEN VERSUS BLUE TETRAZOLIUM CHROMOGEN AS A QUANTITATIVE INDEX OF ADRENOCORTICAL FUNCTION

LEON J. MARKS, M.D., JEHAUDAH H. LEFTIN, B.A. AND MARCIA P. LEONARD, B.A.

The Research Laboratory, Boston Veterans Administration Hospital, Boston, Massachusetts

METHODS thus far published for the estimation of urinary metabolites of the adrenocortical steroids have given only partially satisfactory estimates of adrenocortical function. The most widely used chemical procedure for the measurement of urinary corticosteroids has been the phenylhydrazine-sulfuric acid reaction as introduced by Porter and Silber (1). This reaction is specific for the determination of those cortical steroids which contain both an alpha ketol grouping and a hydroxyl group at the C_{17} position (1–3). Since some of the alpha ketolic corticosteroids elaborated by the human adrenal gland, such as aldosterone and corticosterone (4, 5), do not possess a 17-hydroxyl group, the Porter-Silber reaction cannot be used to measure this class of adrenocortical steroids.

A method for the measurement of alpha ketolic corticosteroids based on the reduction of blue tetrazolium [3,3′-dianisole-bis-4,4′-(3,4-diphenyl)-tetrazolium chloride] in alkaline media was developed by Mader and Buck (6). This reaction does not depend upon the presence of a 17-hydroxyl group and therefore can be used to determine both those steroids which react with the Porter-Silber reagent and those which contain only an alpha ketol grouping at the C_{17} position. Chen and associates have reported that Δ^4-3-keto and 17-α-hydroxy-20-keto groups also exhibit reducing power for blue tetrazolium; but calculated on a molar basis, their power of reduction is only from 5 to 10 per cent of that of alpha ketolic steroids (7). The blue tetrazolium method has been modified for application to urinary extracts by Chen *et al.* (7, 8) and more recently by Sulkowitch and co-workers (9).

It was the purpose of the present study to quantify urinary adrenocortical metabolites as completely as possible by applying both the Porter-Silber and blue tetrazolium reactions to chromatographed urinary extracts from normal adult males and from males with various types of endocrine disease.

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MATERIALS AND METHODS

Urine was collected without preservative in clean bottles. If the determinations could not be performed within twenty-four hours, the specimens were preserved in the frozen state until analyzed. Ambulatory hospitalized men, without evidence of endocrine disease, and male hospital personnel were selected as normal controls. The men selected as controls ranged in age from 21 to 65 years.

A 30-ml. aliquot of urine was hydrolyzed at pH 5.0 with 15,000 Fishman units of β-glucuronidase[1] at 47° C for sixteen hours and then extracted with chloroform and chromatographed on Florisil columns (2). The columns were eluted with chloroform, with 4 per cent methanol in chloroform, and finally with 25 per cent methanol in chloroform. Standard chloroform solutions of cortisone, hydrocortisone, tetrahydrohydrocortisone, and desoxycorticosterone were chromatographed on the Florisil columns and then eluted as described. The first three compounds were found primarily in the 25 per cent methanolic chloroform eluate, whereas desoxycorticosterone was eluted by both the 4 per cent and 25 per cent methanolic chloroform fractions in equal quantities.

Preliminary experiments with normal urine extracts indicated that both the 4 per cent and 25 per cent methanolic chloroform fraction contained significant quantities of blue tetrazolium reducing substances. Consequently, the 4 per cent and 25 per cent methanolic chloroform fractions were collected separately and divided into two equal portions. Half of each fraction was combined and the solvents evaporated. The blue tetrazolium[2] reaction was carried out on this residue according to the method of Chen *et al.*, employing choline and 95 per cent ethanol (7). The other half of the 25 per cent fraction was evaporated and the Porter-Silber reaction applied according to the modification of Reddy *et al.* (3), with ethanol being used as the solvent in place of butanol. The remaining half of the 4 per cent fraction was discarded. 17-Hydroxycorticosterone was used as the standard for both color reactions. Urinary 17-ketosteroids were determined by the Zimmermann reaction, according to a modification of the method of Callow *et al.* (10).

RESULTS

Figure 1 illustrates the range of chromogen excretion in 42 presumably normal adult males. The urinary excretion of Porter-Silber chromogen (hereafter referred to as PSC) ranged between 2.5 mg. and 6.4 mg. per twenty-four hours, with a mean of 4.4 ± 1.2.[3] The urinary excretion of blue tetrazolium chromogen (hereafter referred to as BTC) ranged between 8.8 mg. and 33 mg. per twenty-four hours with a mean of 18.3 ± 7.5. Urinary PSC and BTC excretions appeared to be independent of each other, as indicated by a correlation coefficient of -0.004.

In Table 1 are listed the values for 24-hour urinary excretion of PSC, BTC and 17-ketosteroids in male patients with various endocrine diseases. In patients with primary or secondary adrenocortical insufficiency, low values for all three types of urinary steroids were observed, except for one

[1] β-Glucuronidase was obtained from Warner-Chilcott Laboratories, New York, and is available under the trade name, "Ketodase."

[2] Blue tetrazolium was obtained from Monomer-Polymer, Inc., Leonminster, Mass.

[3] Standard deviation.

normal level of BTC in a patient with Addison's disease. In 1 patient with a large pituitary tumor, who presented no clinical evidence of Cushing's syndrome, moderately elevated values of all urinary steroids were consistently found. Following high voltage roentgen therapy, the urinary steroid pattern fell toward normal levels within one month, and by three months low values for PSC were observed.

In a patient in whom a bilateral orchiectomy and adrenalectomy had been performed for metastatic carcinoma of the prostate, the oral adminis-

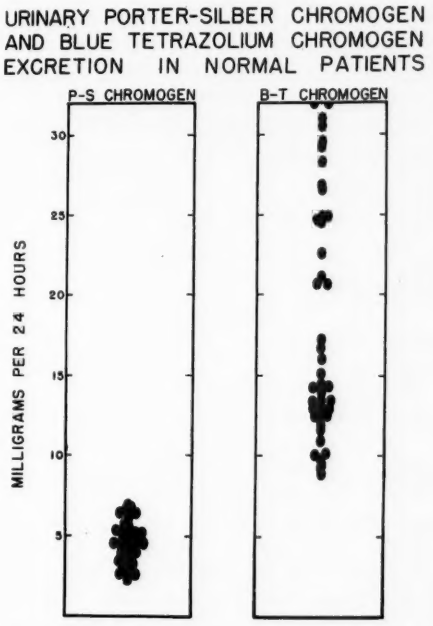

FIG. 1. Range of urinary Porter-Silber chromogen and blue tetrazolium chromogen excretion in normal adult males.

tration of 37.5 mg. of cortisone acetate per day resulted in a urinary PSC excretion of 4.5 to 6.9 mg. and a BTC excretion of 19.1 to 26.4 mg. per twenty-four hours. In this patient, the urinary excretion of PSC accounted for 12 to 19 per cent of the daily oral dose of cortisone acetate, and the excretion of BTC accounted for 58 to 73 per cent of the dose.

Table 2 shows the urinary steroid response to an intravenous infusion of 25 units of corticotropin (ACTH) administered over a six-hour period. The tests were performed on 5 male patients in whom the possibility of adrenal insufficiency was considered clinically. Patients L. S., W. B. and

TABLE 1. URINARY EXCRETION OF PORTER-SILBER CHROMOGEN, BLUE TETRAZOLIUM CHROMOGEN, AND 17-KETOSTEROIDS IN MALE PATIENTS WITH VARIOUS ENDOCRINE DISEASES

Patient	Age	Clinical diagnosis	Porter-Silber chromogen (mg./24 hrs.)	Blue tetrazolium chromogen (mg./24 hrs.)	17-Ketosteroids (mg./24 hrs.)
J.F.	57	Bilateral amyloidosis of adrenals*	0.0	3.5	0.3
J.C.	40	Addison's disease	0.8	17.1	4.6
T.P.	56	Hypopituitarism	1.2	6.5	1.5
F.P.	57	Myotonia dystrophica	1.8	8.4	3.8
R.C.	40	Primary hypogonadism	2.8	16.6	3.2
W.S.	32	Hyperthyroidism with malignant exophthalmos	3.1	12.2	4.5
J.R.	45	Pituitary tumor—			
		before treatment	12.0	63.5	28.4
		1 month after treatment	7.1	42.5	21.2
		3 months after treatment	2.3	22.2	13.5
B.K.	57	Carcinoma of prostate treated by bilateral orchiectomy and adrenalectomy; maintained on 37.5 mg. cortisone acetate orally per day	5.0	19.1	1.3
			6.4	26.4	0.6
			6.9	24.8	1.1
			4.5	21.9	1.2

* Confirmed at autopsy.

F. S. showed evidence of malnutrition and recent moderate weight loss. With 1 exception (G. L.) this group improved markedly without adrenal steroid therapy.

In all 5 patients, urinary BTC excretion rose promptly during administration of corticotropin. In Patients W. B., G. L. and F. S., the increase was much greater than could be accounted for by a corresponding rise in urinary PSC and 17-ketosteroids. No explanation is evident for the poor urinary PSC response in Patient F. S. The low control value for urinary 17-ketosteroids with a corresponding mild increase in response to corticotropin may be related to the presence of chronic malnutrition and/or liver disease in the first 4 patients (11, 12).

TABLE 2. URINARY PORTER-SILBER CHROMOGEN, BLUE TETRAZOLIUM CHROMOGEN, AND 17-KETOSTEROID RESPONSE TO A SIX-HOUR INTRAVENOUS INFUSION OF CORTICOTROPIN (ACTH)

Patient	Age	Clinical diagnosis	Status	Porter-Silber chromogen (mg./24 hrs.)	Blue tetrazolium chromogen (mg./24 hrs.)	17-Ketosteroids (mg./24 hrs.)
L.S.	50	Bronchiectasis	Control	3.1	33.2	1.5
		Bronchopneumonia	ACTH	16.5	54.3	2.0
W.B.	41	Arrested pulmon. tbc.	Control	2.4	26.6	2.1
		Emphysema	ACTH	7.7	44.0	4.4
		Cirrhosis of liver				
G.L.	55	Arrested pulmon. tbc.	Control	1.8	19.1	2.7
		Pulmonary fibrosis	ACTH	2.5	29.9	4.5
		Cor pulmonale	ACTH	6.9	88.1	6.7
		Cirrhosis of liver*	Control	2.4	12.2	3.5
F.S.	59	Depression	Control	1.0	10.3	6.1
		Emphysema	ACTH	2.5	43.1	9.1
		Malnutrition	ACTH	3.5	45.7	12.0
P.D.	53	Hypothyroidism	Control	3.5	31.2	14.3
			ACTH	16.5	47.5	19.4

* Moderately severe hemorrhagic infarction of both adrenals found at autopsy (*see* text).

The minimal urinary PSC response in Patient G. L. may have been related to the presence of a bilateral adrenal lesion. This patient, who had diffuse pulmonary fibrosis and cor pulmonale, became semi-comatose and disoriented one week before his sudden demise. The adrenocortical function studies were completed thirty-six hours before his death. Autopsy confirmed the clinical diagnoses, but also revealed moderately severe hemorrhagic infarction of both adrenal glands.

In an endeavor to test the specificity of both urinary PSC and BTC as measures of adrenal corticosteroids, the following study was performed. A patient without testicular or adrenal tissue who was being maintained on oral cortisone acetate, was treated with 1 mg. of 9α-fluorohydrocortisone acetate[4] daily. The 4 per cent and 25 per cent methanolic chloroform fractions were collected separately and divided in half. Both PSC and BTC

[4] Florinef acetate, E. R. Squibb and Sons, New York.

were determined in each fraction. A dose of 40 units of Zinc-ACTH[5] was administered parenterally once a day for six days. On the seventh day, a dose of 150 mg. of hydrocortisone was administered orally.

The results of this study may be seen in Figure 2. While the patient was maintained on cortisone acetate, the PSC of the 4 per cent fraction amounted to 15 per cent of the PSC in the 25 per cent fraction. Once fluorohydrocortisone acetate was substituted for cortisone acetate, urinary PSC promptly disappeared. Administration of Zinc-ACTH did not increase

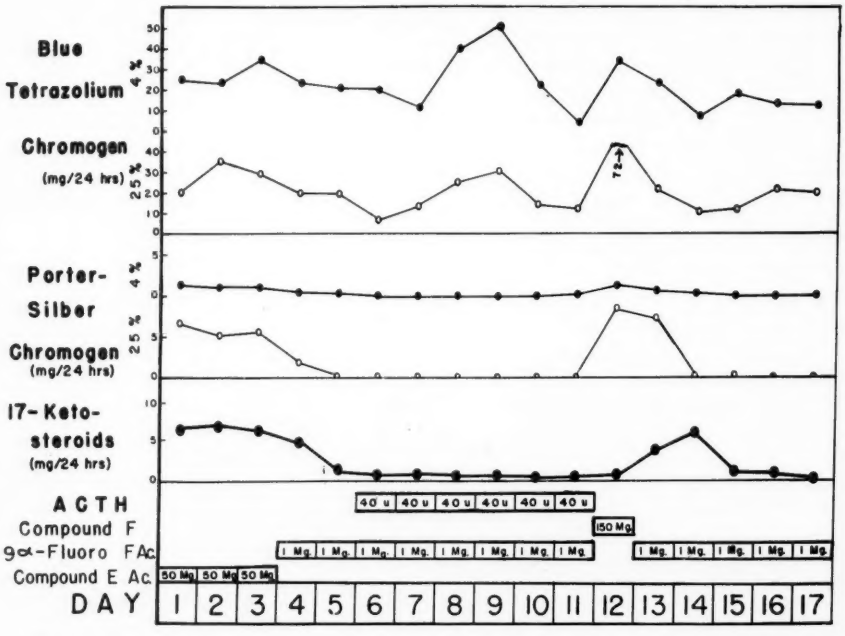
Blue Tetrazolium 4%
Chromogen (mg/24 hrs) 25%
Porter-Silber Chromogen (mg/24 hrs)
17-Keto-steroids (mg/24 hrs)
ACTH
Compound F
9α-Fluoro FAc.
Compound E Ac.
DAY
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

FIG. 2. Daily variation in urinary excretion of Porter-Silber chromogen and blue testrazolium chromogen in a 57-year-old man without testicular or adrenal tissue, in response to the administration of various adrenocortical corticosteroids orally and Zinc-ACTH parenterally.

urinary PSC. Hydrocortisone given orally resulted in a prompt increase in urinary PSC excretion, which lasted forty-eight hours.

Urinary BTC, on the other hand, was well maintained in both fractions during all phases of the study. There were marked fluctuations in urinary BTC but, in general, administration of hydrocortisone appeared to effect an increase in BTC excretion for a short period.

[5] Kindly supplied by Organon, Inc., Orange, New Jersey.

DISCUSSION

Our range of normal urinary values for 17-hydroxycorticosteroids is in agreement with that reported by Sandberg *et al.* (13), who employed the original Glenn-Nelson method. However, our absolute values are somewhat lower. This difference is probably related to the fact that in the application of the Porter-Silber reaction to urinary extracts we employed a ratio of 4:1 of reagent to solvent, whereas Glenn and Nelson used a ratio of 3:2 of reagent to solvent. In our experience employing the same concentration of hydrocortisone as a standard, the higher the ratio of reagent to solvent, the lower the resulting PSC value of the urinary extract (14).

Our normal values for urinary blue tetrazolium reducing corticosteroids are approximately ten times higher than those previously reported by Wheeler *et al.* (8) and three to four times higher than the results of Sulkowitch *et al.* (9). The first of these differences may be partially explained by the fact that Wheeler *et al.* employed acid hydrolysis of urine rather than β-glucuronidase hydrolysis. In Sulkowitch's modification of the blue tetrazolium reaction for the quantitative assay of corticosteroids, desoxycorticosterone or hydrocortisone react only half as intensely as equivalent amounts of cortisone or dehydrocorticosterone. With Chen's method of assay, the reducing power of different adrenocortical hormones with a ketol side chain is, mole for mole, essentially the same. Sulkowitch *et al.* expressed their results in milligrams of cortisone, whereas we used hydrocortisone as a standard. Secondly, Sulkowitch *et al.* employed 150 Fishman units of β-glucuronidase per cubic milliliter of urine, whereas we employed 500 Fishman units per cubic milliliter of urine. Glenn and Nelson (2) have recommended a minimal β-glucuronidase concentration of 330 Fishman units per cubic milliliter of urine in order to insure complete hydrolysis of steroid glucuronides.

When urinary PSC was compared with urinary BTC following β-glucuronidase as a quantitative index of adrenocortical function, it was found that BTC excretion was greater than PSC excretion in every patient. The difference between urinary BTC and PSC in normal adult males ranged from 4.5 to 27.0 milligrams per twenty-four hours. A correlation coefficient close to zero indicated that there was no correlation between urinary PSC and BTC excretions as measurements of adrenocortical function in normal males.

As illustrated in Table 2, there was a poor correlation between the response of urinary PSC and the response of urinary BTC to the intravenous administration of corticotropin (ACTH). Whereas there was a marked rise in urinary BTC in all 5 of the chronically ill patients, urinary PSC did not increase significantly in 2 of them. In 1 of these 2, autopsy revealed bilateral hemorrhagic adrenal infarction.

Inasmuch as the correlation between urinary PSC excretion and BTC excretion was poor in normal adult males, in patients with endocrine disease, and in men given corticotropin intravenously, it seemed important to test the specificity of both urinary PSC and BTC as measures of adrenocortical function in an adult male without testicular or adrenal tissue. When this patient received 1 milligram of 9-α-fluorohydrocortisone acetate per day, urinary PSC promptly disappeared, but urinary BTC persisted at a slightly reduced level. In light of these results, it is doubtful that urinary BTC is as specific a measure of urinary adrenocortical metabolites as is urinary PSC, and consequently could be quite misleading in the study of spontaneous alterations in adrenocortical function in various disease states.

SUMMARY

1. The daily urinary excretions of Porter-Silber chromogen and blue tetrazolium chromogen have been determined by a method employing β-glucuronidase hydrolysis and chromatography in (a) normal adult males, (b) male patients with endocrine diseases, and (c) male patients following the administration of corticotropin.

2. The correlation between urinary Porter-Silber chromogen and urinary blue tetrazolium chromogen as measurements of adrenocortical function was poor in all three classes of patients studied.

3. In a patient without testicular or adrenal tissue, a maintenance dose of 1 milligram of fluorohydrocortisone acetate daily resulted in the prompt disappearance of urinary Porter-Silber chromogen, but urinary blue tetrazolium chromogen persisted at a slightly reduced level.

4. Urinary Porter-Silber chromogen has been shown to be a more specific quantitative measurement of adrenocortical function in man than urinary blue tetrazolium chromogen.

Acknowledgment

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THE EFFECT OF PHENYLBUTAZONE AND A RELATED ANALOGUE (G25671) UPON THYROID FUNCTION*

JOSEPH A. LINSK, M.D.,† BRUCE C. PATON, M.D., MURRAY PERSKY, M.D., MARION ISAACS, M.D. AND HERBERT S. KUPPERMAN, M.D., PH.D.

New York University College of Medicine (Third Medical Division), Goldwater Memorial Hospital and Department of Therapeutics, New York University-Bellevue Medical Center, New York, N. Y.

INTRODUCTION

PHENYLBUTAZONE is a pyrazalone derivative which has been shown to have effects similar to those of cortisone and corticotropin with respect to anti-rheumatic activity and sodium retention (1). In 1952 Green *et al.* demonstrated that, in addition to its other pharmacologic effects, phenylbutazone had a marked inhibiting action on the uptake of I^{131} by the thyroid (2); this followed the daily administration of 1 gram of the drug for two or three days. It was suggested by Scott *et al.* (3), on the basis of work in rats, that phenylbutazone combines with iodine in the body and thus prevents it from being taken up by the thyroid. It was also observed that, despite continuing administration of the drug, thyroidal iodine uptake in the rat returned towards normal when phenylbutazone was given for more than ten days. This was attributed to a saturation point following which the phenylbutazone could no longer bind the iodine and thyroidal accumulation of iodine then returned to normal levels.

The present study was designed to confirm the previous findings in man on the effect of phenylbutazone upon the uptake of I^{131} by the thyroid, and to correlate this effect with the attained plasma level of phenylbutazone. Additional studies were carried out in an attempt to determine the possible mechanisms of action. An analogue of phenylbutazone, namely, 4-(phenylthioethyl)-1,2-diphenyl-3,5-pyrazolidinedione or (G-25671), was also tested for its effects on thyroidal I^{131} uptake.

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† Present address: 4021 Atlantic Avenue, Atlantic City, N. J.

METHODS

Thirteen euthyroid patients were used for the study. In all 13, a control 24-hour thyroidal I^{131} uptake after oral I^{131} administration was determined at least one week prior to the administration of phenylbutazone and G-25671.

The dose of I^{131} was 7.0 to 30.0 microcuries. Counts were made at the following distances: 12 inches from the thyroid and 6 inches from the thigh. The percentage thyroidal uptake was calculated with reference to a standard consisting of a 25-cc. volumetric flask containing the same dose of I^{131} as the patient had received. Counts were made at a distance of 12 inches from this flask at the same time as counts were made over the patients' thyroids.

Phenylbutazone was administered orally in a daily dose of 800 mg. for four days. On the morning of the fourth day, at least one hour before breakfast, the I^{131} was given. Twenty-four hours later the counts were made and, at the same time, a blood sample taken for estimation of the plasma level of the phenylbutazone. Plasma phenylbutazone levels were calculated according to the method described by Burns *et al.* (4). The thyroidal uptake of I^{131} was recalculated in 3 patients one week after phenylbutazone therapy was stopped.

This procedure was repeated in 4 patients, using G-25671 instead of phenylbutazone. The total daily amount of G-25671 was 1,400 mg. administered in three doses. Such a division was necessary because it has been shown by Brodie *et al.* (5) that this drug has a physiologic half-life of only three hours and frequent doses are required in order to maintain adequate plasma levels. For comparison, the biologic half-life of phenylbutazone is seventy hours (5). I^{131} uptake was determined on the morning of the fourth day of G-25671 therapy.

The acute effect of phenylbutazone was also studied in 3 patients after intravenous administration of the drug. The rate of thyroidal I^{131} uptake was first determined over a 10-hour period. At a later date the same patients were given simultaneously 800 mg. of phenylbutazone intravenously and a dose of I^{131} orally. The rate of thyroidal I^{131} uptake was then re-determined, counts being made at intervals of one to two hours for ten hours.

An investigation of the duration of effect of phenylbutazone upon thyroidal I^{131} uptake was undertaken after sixteen to twenty-one days of continuous administration of the drug to 4 patients. In 2, the uptake was also determined after ninety-seven days of uninterrupted medication.

A study of the influence of thyroid-stimulating hormone (TSH) and phenylbutazone was also undertaken in 3 patients. After establishing their thyroidal I^{131} uptake during phenylbutazone therapy, they were given phenylbutazone together with 10 units of TSH subcutaneously every day. The thyroidal uptake of I^{131} was established on the fourth day of combined therapy.

RESULTS AND DISCUSSION

A consistent and marked reduction in thyroidal uptake of I^{131} was seen in all 13 patients (Table 1, Fig. 1). This was found in the presence of plasma levels of phenylbutazone which fell within the usual therapeutic range throughout the period of the study.

The physiologic distribution and pharmacologic actions of G-25671 have recently been reported. It is an analogue of phenylbutazone with a phenyl-

TABLE 1. EFFECT OF PHENYLBUTAZONE AND G-25671 UPON THYROIDAL I^{131} UPTAKE

Patient	Control thyroidal I^{131} uptake	After 4 days of phenylbutazone therapy (800 mg./day)			After omission of phenylbutazone for 7 days			Phenylbutazone (800 mg./day) +TSH (10 units/day) for 4 days			After 4 days of G-25671 therapy (1400 mg./day)			Continuation of phenylbutazone therapy (800 mg./day)			
		Thyroidal I^{131} uptake	Per cent of control uptake	Blood phenylbutazone level (μg./ml.)	Thyroidal I^{131} uptake	Per cent of control uptake	Blood phenylbutazone level (μg./ml.)	Thyroidal I^{131} uptake	Per cent of control uptake	Blood phenylbutazone level (μg./ml.)	Thyroidal I^{131} uptake	Per cent of control uptake	Blood phenylbutazone level (μg./ml.)	Thyroidal I^{131} uptake	Per cent of control uptake	Blood phenylbutazone level (μg./ml.)	Duration of therapy (days)
A.D.	31.3	5.0	16.0	145.0				28.0	89.4	130.0							
B.H.	36.2	6.0	16.5	125.0				29.0	80.1	110.8							
N.K.	21.0	7.8	37.1	107.6	26.5	126.2	32.9	35.0	166.6	135.7							
H.O.	26.4	5.0	19.0	—	28.1	106.5											
M.N.	45.0	6.5	14.4	115.1	36.6	81.3	35.3				39.7	88.2	86.7				
G.S.	49.9	10.3	20.6	—							63.4	127.0	125.9	26.4	53.0	137.9	21
														43.4	85.1	124.5	97
J.McS	41.3	13.2	31.0	—							46.1	111.0	81.4	21.2	51.3	105.2	21
														24.6	59.5	100.4	97
F.K.	20.5	6.5	31.7	133.3							29.0	141.4	125.5				..
O.P.	54.8	13.3	24.3	—										59.1	108.0	82.3	19
J.M.	28.6	7.0	24.4											11.0	38.6	88.0	16
W.S.	35.0	12.1	35.0	123.0													
W.M.	39.5	4.2	10.5	113.0													
G.N.	32.1	9.6	30.0	132.0													

thio-ethyl side-chain instead of a butyl group (Fig. 2). It has some anti-inflammatory action and is a powerful uricosuric agent, but does not induce sodium retention. In this study, it caused no depression of I^{131} uptake by the thyroid, although in the same patients the uptake was markedly depressed by phenylbutazone (Fig. 1, Table 1). The plasma levels of G-25671 were of the same order as those noted for phenylbutazone.

In other reported studies the uptake of I^{131} was determined after phenylbutazone had been administered intramuscularly for two or three days,

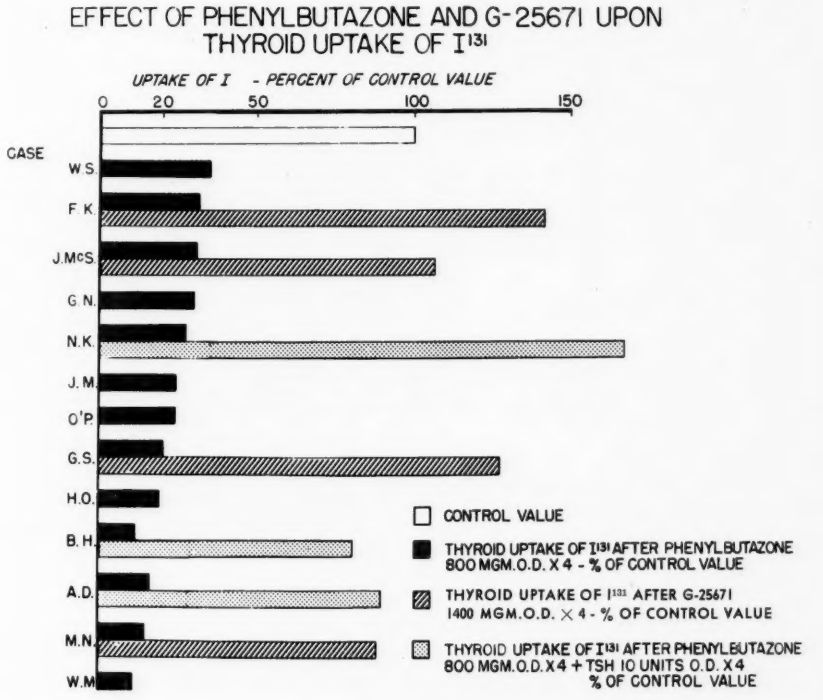
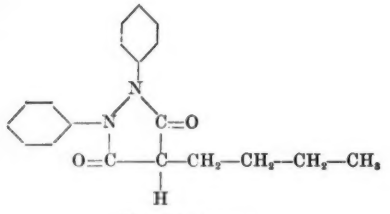


FIGURE 1

and in this study it was determined after the drug had been administered orally for four days. It was therefore decided to investigate the immediate effect of intravenous phenylbutazone upon the I^{131} uptake by giving the oral tracer dose of I^{131} and the intravenous dose of phenylbutazone simultaneously and estimating the rate of uptake over a 10-hour period. This was done in 3 patients, using each patient as his own control. The thyroidal I^{131} uptake was not suppressed (Fig. 3). This would suggest that intravenously administered phenylbutazone does not have an immediate effect

upon the binding of I^{131}. Suppression of I^{131} uptake, however, has been noted following intravenous administration of other preparations, such as hydrocortisone.

In 3 cases the uptake of I^{131} was re-determined one week after the phenylbutazone had been stopped. In 2 cases the uptake, previously reduced, had returned to normal; in 1 case it was still moderately reduced (Fig. 4). The plasma levels of phenylbutazone had dropped from about 110.0 mg. to about 30.0 mg. per liter (Table 1).



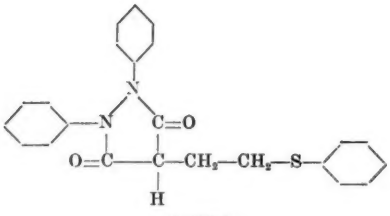


FIGURE 2

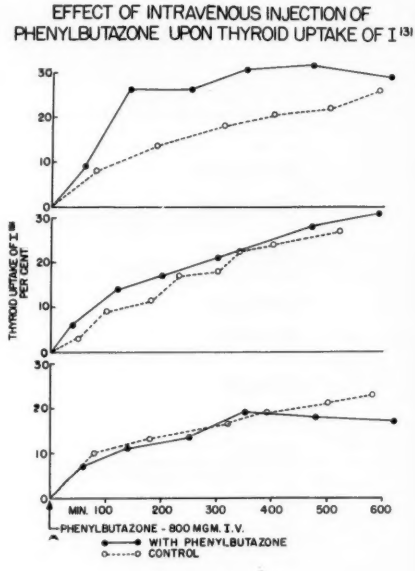


FIGURE 3

This observation on the effect of phenylbutazone upon thyroidal I^{131} uptake may be compared with that on the effect of cortisone (6). In doses of 100 mg. daily, cortisone was found "capable of inhibiting markedly the accumulation of iodine by the normal thyroid gland." When the administration of cortisone was stopped, the I^{131} uptake returned to normal within a few days.

A mechanism whereby phenylbutazone affects the physiologic activity of the thyroid may be a direct depressing action. Determination of the possible mechanism requires studies in which the phenylbutazone content of thyroid tissue can be calculated. However, many of our patients have now received phenylbutazone for more than a year, but no cases of hypothyroidism and no goitrogenic effects have been reported. The reason for

this is apparent from Table 1. When the administration of phenylbutazone is continued for more than four days, the inhibiting action on thyroidal I^{131} uptake wears off, but a partial effect is maintained even up to ninety-seven days (Fig. 5, Table 1). Since only minute amounts of iodine are required for synthesis of thyroxine (7), apparently enough is taken up for this purpose. This action is different from those of cortisone and corticotropin (ACTH), which maintain their properties of inhibiting thyroidal I^{131} uptake

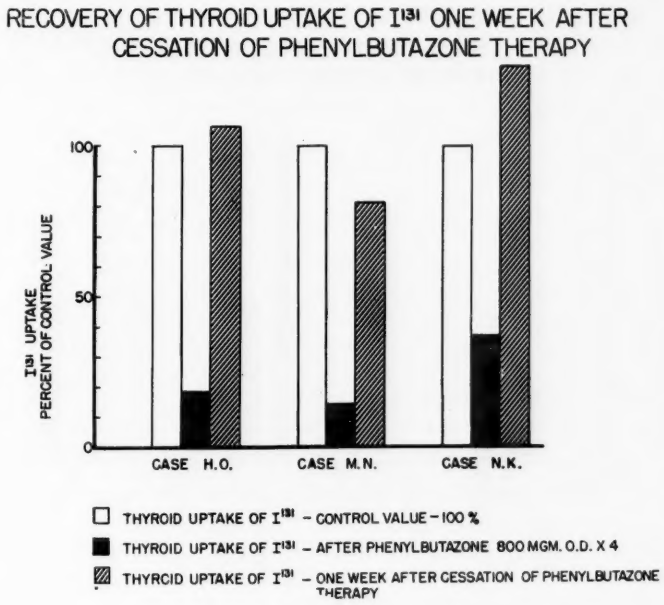

RECOVERY OF THYROID UPTAKE OF I131 ONE WEEK AFTER CESSATION OF PHENYLBUTAZONE THERAPY
I131 UPTAKE PERCENT OF CONTROL VALUE
100
50
0
CASE H.O.
CASE M.N.
CASE N.K.
THYROID UPTAKE OF I131 – CONTROL VALUE – 100 %
THYROID UPTAKE OF I131 – AFTER PHENYLBUTAZONE 800 MGM. O.D. X 4
THYRCID UPTAKE OF I131 – ONE WEEK AFTER CESSATION OF PHENYLBUTAZONE THERAPY

FIGURE 4

as long as they are administered, and may induce some degree of hypothyroidism (8, 9).

Phenylbutazone may depress the output of TSH and thus induce secondary depression of thyroidal activity. It has been suggested that the action of cortisone on I^{131} uptake results from depression of the anterior pituitary secretion of TSH (6). That either cortisone or phenylbutazone can directly inhibit the action of TSH after it has been released from the pituitary is unlikely. This has been demonstrated by our study in which both phenylbutazone and TSH were administered simultaneously for four days to 3 patients. In each case the depressant effect of phenylbutazone on thyroidal I^{131} uptake was nullified by the administration of TSH (Fig. 1,

Table 1). It has been suggested that one effect of TSH is to enhance intracellular enzymatic function in order to facilitate the conversion of inorganic iodine to organic iodine and then to promote increased secretion of the thyroid hormone (7). TSH also increases the uptake of I^{131} by inducing a secondary thyroidal avidity for iodine. Administration of TSH results in an increased uptake of I^{131}, in the face of corticoid inhibition (9).

Since phenylbutazone has a notable effect on electrolytes, it is possible that it accelerates the excretion of iodine in the urine, the feces, or both.

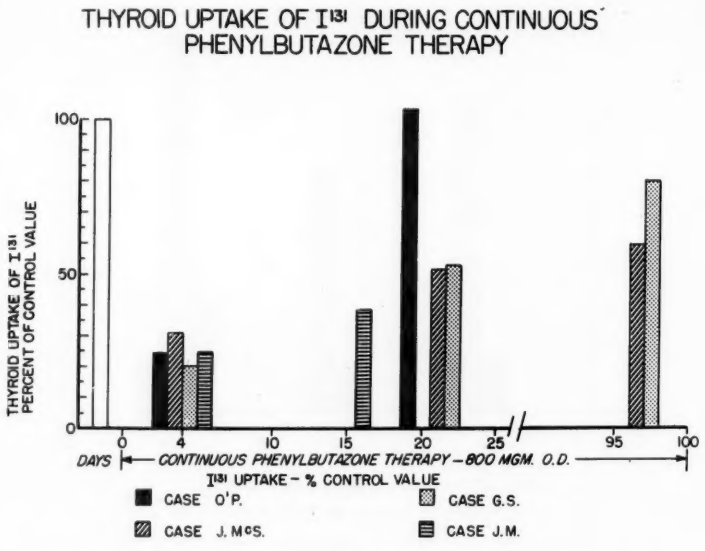


FIGURE 5

Proof of this will depend on the results of quantitative excretion studies in treated myxedematous patients (essentially athyroidal). Iodine excretion studies on patients with intact thyroids would fail to distinguish between primary acceleration of excretion and increased excretion secondary to some block at the thyroid level.

That phenylbutazone directly inhibits TSH in the bloodstream is unlikely, in view of the data. Although both phenylbutazone and cortisone under certain conditions interfere with thyroidal I^{131} uptake, it appears that phenylbutazone, unlike cortisone, induces a temporary or partial suppression of TSH action, thereby decreasing I^{131} uptake during initial therapy with phenylbutazone. This suppression, in contrast to that induced by cortisone, is not effective during prolonged therapy with phenylbutazone.

SUMMARY

Administration of phenylbutazone results in a reduction of thyroidal I^{131} uptake.

G-25671, a structural analogue of phenylbutazone possessing anti-inflammatory and uricosuric activity, does not interfere with I^{131} uptake.

Phenylbutazone and cortisone have several known parallel effects. Both drugs are anti-rheumatic, ulcerogenic and uricosuric; both cause sodium retention. Two further parallel effects can now be added. They both depress the thyroidal uptake of I^{131}, and in both instances this depression is apparently neutralized by the administration of TSH. However, the effect of phenylbutazone is temporary despite continued therapy, whereas that of cortisone is maintained as long as cortisone is administered.

Within the limits of the studies reported here, it appears that, of the possible explanations for the lowering of thyroidal I^{131} uptake by phenylbutazone, depression of pituitary function is the most likely.

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CORRELATION OF EARLY EXTRATHYROIDAL RADIOIODIDE SPACE WITH BODY WEIGHT

ROSALYN S. YALOW, PH.D., ESTELLE S. SOBEL, M.S. AND KARL R. PALEY, M.D.

The Isotope Laboratory of the Lenox Hill Hospital, New York, and the Radioisotope Service of the Veterans Administration Hospital, Bronx, N. Y.*

THE thyroidal iodide clearance rate is a direct measure of the iodide accumulating function of the thyroid gland. It expresses the rate of thyroidal iodide uptake divided by the mean plasma iodide concentration measured simultaneously. In this laboratory the method of Berson *et al.* (1) is used for routine determinations of thyroidal iodide clearance rates. The thyroidal I^{131} uptake is measured during the first half hour following intravenous administration of the tracer dose, with suitable corrections for changes in extrathyroidal radioactivity during this interval. The direct determination of the mean plasma radioiodide concentration would require multiple plasma assays, but in this method it is approximated as that concentration which would result if the apparent volume of distribution of I^{131} (exclusive of thyroidal and urinary accumulation) averaged 20 per cent of the body weight for this initial half-hour period ("mean iodide space"). This value for the mean iodide space was determined experimentally by Berson *et al.* (1) in a group composed principally of male patients. Thyroid disease is, however, more common in the female population by a ratio of 4 to 1 (2). Since total body water (3), plasma volume (4), and extracellular space (5) are smaller fractions of body weight in women than in men, it might be anticipated that the mean half-hour iodide space would also show a significant sex difference.

In this study, the mean radioiodide spaces of distribution during the first half hour following intravenous administration for male and female euthyroid subjects in the normal weight range were compared with the values obtained by Berson *et al.* (1). The mean spaces were also determined for grossly overweight and underweight subjects and for hyperthyroid subjects within the normal weight range.

METHODS

A. *Subjects*

Patients referred to the Isotope Laboratory at Lenox Hill Hospital served as subjects for this study. These included 11 euthyroid and 3 hyperthyroid male subjects and 20

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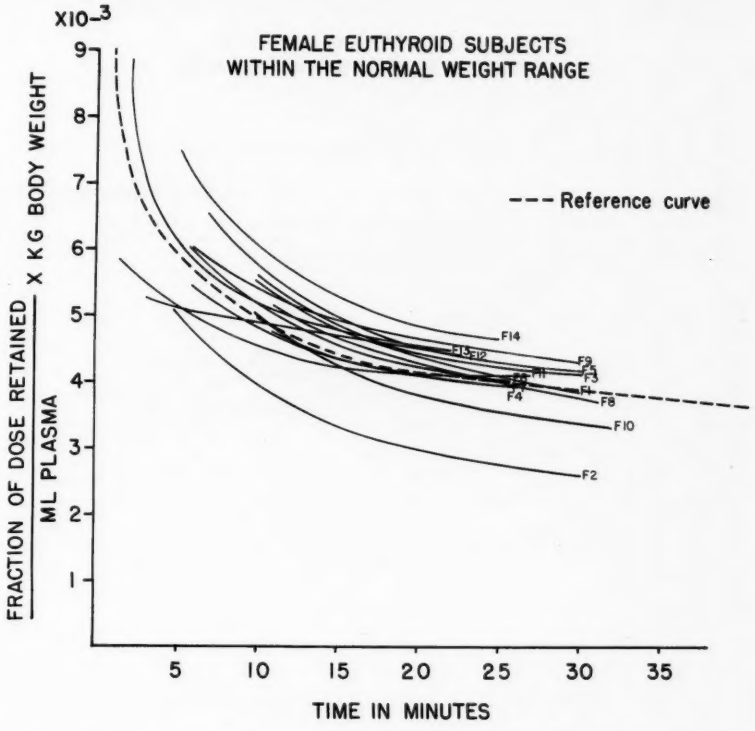

FIG. 1 a. Venous plasma I^{131} concentration curves as a function of time for female euthyroid subjects within the normal weight range.

euthyroid, 1 hypothyroid and 5 hyperthyroid female subjects. Three of the men and 8 of the women were outside the normal weight range.[1]

B. *Procedure*

A dose of 25 to 58 microcuries of radioiodine was administered intravenously, following which the radioactivity over the neck region was assayed at one-minute intervals for thirty minutes. During this period several venous blood samples were drawn and at the end of the time a complete urinary collection was obtained. The thyroidal radioactivity was assayed using a bismuth-walled Geiger counter placed 23 cm. from the neck. The sensitivity of this arrangement was 103 counts per minute per microcurie of I^{131} above a background of 60 counts per minute, as determined with a phantom neck consisting of a 600-ml. beaker filled with paraffin and containing a source of I^{131} resembling the thyroid in size and location. For continuous recording, the register output of the scaling circuit was connected in parallel with a Streeter-Amet printing register. Radioactivities

[1] Normal weight ranges for given heights and ages were obtained from Metropolitan Life Insurance Tables.

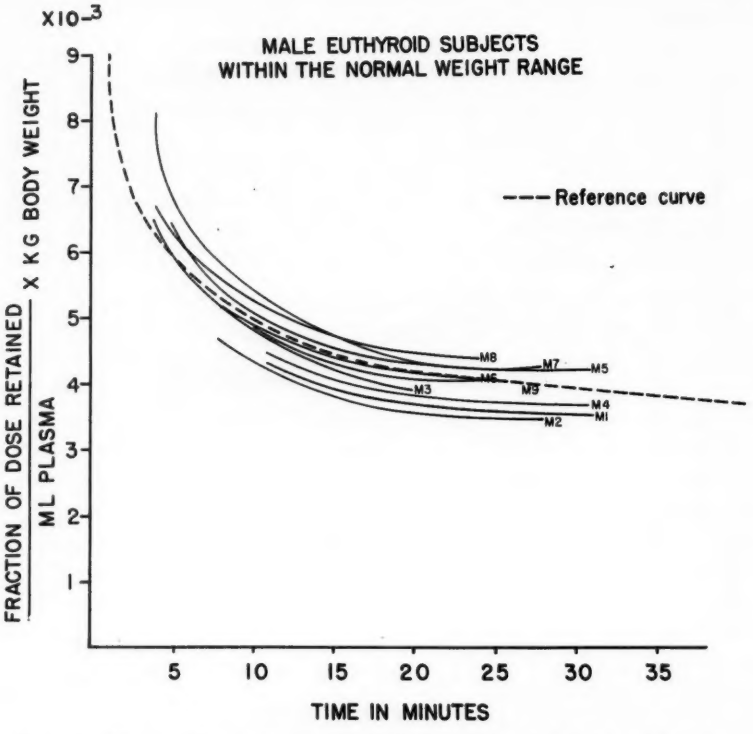

FIG. 1 b. Venous plasma I^{131} concentration curves as a function of time for male euthyroid subjects within the normal weight range.

in the administered dose and in urine were assayed in Marinelli beakers placed around a bismuth-walled Geiger counter with a sensitivity of 5.35×10^3 counts per minute per microcurie of I^{131} above a background of approximately 40 counts per minute. Radioactivities in 5-ml. plasma samples were assayed in a well-type scintillation counter with a sensitivity of 1.00×10^6 counts per minute per microcurie of I^{131} over a background of approximately 240 counts per minute.

The thyroidal radioactivity at any time is calculated from the assay of total radioactivity over the neck by a method previously described (1). The cumulative urinary excretion at the same time is estimated approximately from the half-hour excretion, assuming that the renal clearance rate remained constant during the thirty-minute period. Since, in all patients, the half-hour urinary excretion was less than 10 per cent of the administered radioactivity, even moderate variations in the rate of the renal clearance would not produce a significant error in the determination of the extrathyroidal radioactivity remaining in the body.

C. *Analysis of data*

In order to relate plasma concentration to body size, a linear plot was made of the product of body weight times the fraction of the extrathyroidal radioactivity per ml. of

TABLE 1

Patient	Age (yrs.)	Weight (Kg.)	Height (ins.)	Relative area*
A. *Euthyroid female subjects within the normal weight range*				
F 1 E.Mo.	63	64	66	1.06
F 2 M.Mun.	22	56	67	.76
F 3 I.N.	47	60	66	1.08
F 4 E.R.	32	71	69	.99
F 5 A.C.	39	55	60	1.12
F 6 M.D.	24	60	66	1.02
F 7 J.H.	41	67	64	.98
F 8 E.Ma.	53	63	59	1.01
F 9 L.C.	50	56	60	1.10
F 10 C.N.	31	56	62	.94
F 11 E.C.	46	59	67	.93
F 12 E.Z.	34	62	67	.98
F 13 S.T.	47	50	62	1.07
F 14 J.V.	25	68	68	1.19
				1.02 ± .10

Mean relative area = $1.02 \pm .10$

Mean $\frac{1}{2}$-hr. space of dilution = $\frac{20\%}{1.02 \pm .10} = (20 \pm 2)\%$ body wt.

B. *Euthyroid male subjects within the normal weight range*				
M 1 L.B.	37	87	71	.87
M 2 L.L.	59	82	66	.85
M 3 W.S.	65	86	70	.95
M 4 C.S.	53	69	68	.91
M 5 J.T.	44	75	73	1.17
M 6 B.H.	65	80	71	1.00
M 7 G.T.	26	86	70	1.07
M 8 P.F.	26	74	68	1.09
M 9 J.P.	53	73	72	1.00
				.99 ± .10

Mean relative area = $.99 \pm .10$

Mean $\frac{1}{2}$-hr. space of dilution = $\frac{20\%}{.99 \pm .10} = (20 \pm 2)\%$ body wt.

* Relative area = $\frac{\text{Area under dilution curve of patient}}{\text{Area under the reference curve (1)}}$ for same time interval.

plasma as a function of time (Figs. 1, 2 and 3). A smooth curve connecting all experimental points was drawn for each subject. The concentration curve was not experimentally determined for the initial few minutes following administration. Therefore, for each patient, the area under the experimentally determined plasma concentration curve (for the total time interval during which venous samples were obtained) was compared with the area for the same time interval under the composite similar curve of Berson *et al.*, hereafter termed the "reference curve" (Tables 1, 2 and 3). The mean values and the standard deviations for the ratios of these areas to the area under the reference curve ("relative areas") were calculated separately for male and female euthyroid subjects whose weights were within the normal range. The mean values for the relative areas were obtained for overweight and underweight subjects regardless of thyroid status, and for hyperthyroid subjects in the normal weight range.

The reciprocal value of the area under a concentration curve for a given time interval represents the mean extrathyroidal space of iodide distribution during that time interval as a function of body weight. Therefore, dividing the mean half-hour radioiodide space obtained from the reference curve (20 per cent of body weight) by the mean relative area for each group yields the mean half-hour space for that group (Tables 1, 2 and 3).

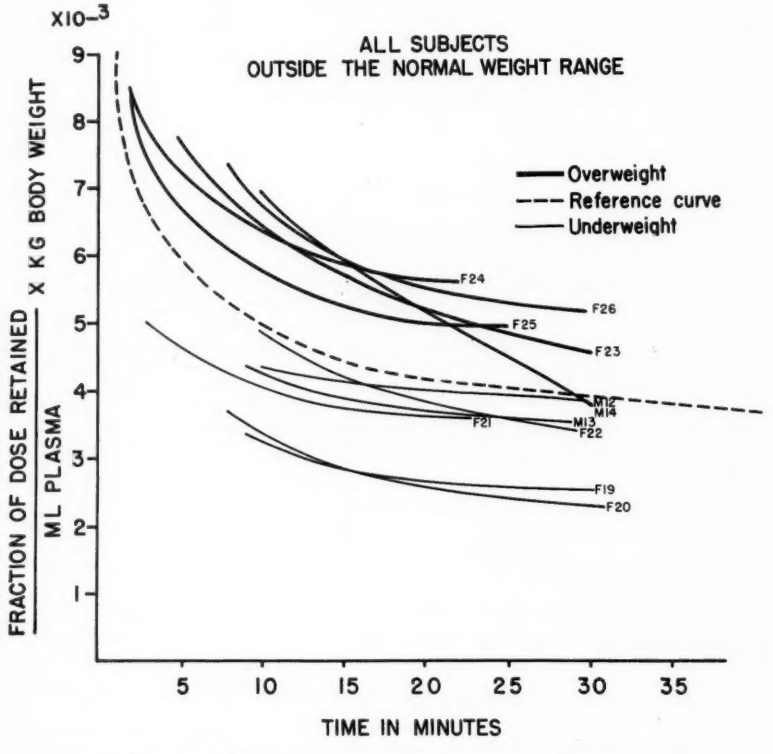

FIG. 2. Venous plasma I^{131} concentration curves as a function of time for subjects outside the normal weight range.

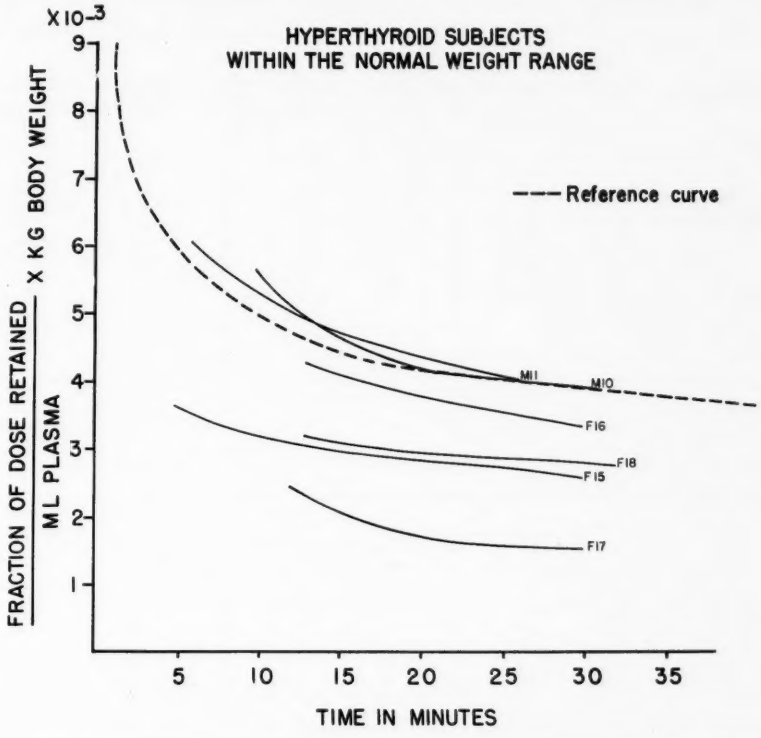

FIG. 3. Venous plasma I^{131} concentration curves as a function of time for hyperthyroid subjects within the normal weight range.

For each of the overweight, underweight and hyperthyroid subjects the ratio of the patient's weight to the average normal weight for his or her height and age was also determined. This ratio was then employed as a correction factor for the relative area in order to evaluate the radioiodide space of distribution in terms of the average normal body weight for the individual subject, rather than the actual weight at the time of the test (Tables 2 and 3).

RESULTS

For both male and female euthyroid subjects within the normal weight range, the mean radioiodide space of dilution for the half-hour period following intravenous administration of I^{131} differed from the value given by Berson *et al.* (1) by less than 2 per cent. These differences are not significant, since they are less than the standard errors of the means.

For both female and male euthyroid subjects within the normal weight range, the half-hour space of dilution of radioiodide was (20 ± 2) per cent of the body weight (Table 1, Fig. 1). For all subjects outside the normal

TABLE 2

Patient	Thyroid status	Age (yrs.)	Weight (Kg.)	Height (ins.)	Relative area*	Avge. normal wt. for ht. and age (Kg.)	Relative weight†	Relative area / Relative weight
				A. *Overweight subjects*				
F 23 M.W.	Eu	50	85	63	1.25	64	1.33	.94
F 24 C.A.	Eu	26	85	62	1.29	57	1.49	.86
F 25 D.G.	Eu	28	66	62	1.23	57	1.16	1.06
F 26 G.G.	Hypo	68	97	60	1.32	61	1.59	.83
M 14 W.Z.	Eu	48	97	70	1.27	80	1.21	1.04
					1.27		*1.35*	*.95 ± .09*

Mean relative area = 1.27.

Mean ½-hr. space of dilution = $\frac{20\%}{1.27}$ = 16% body wt.

Mean $\frac{\text{relative area}}{\text{relative weight}}$ = .95 ± .09.

Mean ½-hr. space of dilution = $\frac{20\%}{.95 \pm .09}$ = (21 ± 2)% avge. normal body wt. for ht. and age.

				B. *Underweight subjects*				
F 19 L.H.	Eu	78	48	63	.63	65	.74	.85
F 20 K.K.	Eu	50	54	67	.63	72	.75	.84
F 21 M.Mul.	Eu	55	40	61	.83	62	.65	1.28
F 22 M.N.	Hyper	57	51	64	.91	66	.77	1.18
M 12 R.M.	Eu	52	57	70	.97	78	.73	1.32
M 13 T.U.	Hyper	42	66	76	.86	91	.73	1.18
					.80		*.73*	*1.11 ± .18*

Mean relative area = .80.

Mean ½-hr. space of dilution = $\frac{20\%}{.80}$ = 25% body wt.

Mean $\frac{\text{relative area}}{\text{relative weight}}$ = 1.11 ± .18.

Mean ½-hr. space of dilution = $\frac{20\%}{1.11 \pm .18}$ = (18 ± 3)% avge. normal body wt. for ht. and age.

* Relative area = $\frac{\text{Area under dilution curve of patient}}{\text{Area under the reference curve (1)}}$ for same time interval.

† Relative weight = $\frac{\text{Wt. of subject}}{\text{Average normal wt. for ht. and age}}$.

weight range, the mean half-hour space of radioiodide distribution appeared to be better correlated with the average weight for the subject's height and age than with the actual weight (Table 4). Hyperthyroid subjects within the normal weight range had lost on the average 14 per cent of the body weight in the course of their disease. For this reason, their mean radioiodide space was also better correlated with the average weight for their height and age than with their actual weight (Table 4).

TABLE 3. HYPERTHYROID SUBJECTS WITHIN THE NORMAL WEIGHT RANGE

Patient	Age (yrs.)	Weight (Kg.)	Height (ins.)	Relative area*	Avge. normal wt. for ht. and age (Kg.)	Relative weight†	Relative area / Relative weight
F 15 F.S.	30	48	64	.65	60	.80	.82
F 16 G.M.	55	58	62	.89	64	.91	.98
F 17 A.B.	39	47	62	.45	59	.80	.57
F 18 G.K.	37	54	63	.72	62	.87	.83
M 10 M.B.	62	56	63	1.04	65	.86	1.21
M 11 J.B.	54	76	70	1.04	75	1.01	1.03
				.86		.88	.91 ± .19

Mean relative area = .80.

Mean ½-hr. space of dilution $= \frac{20\%}{.80} = 25\%$ body wt.

Mean $\frac{\text{relative area}}{\text{relative weight}} = .91 \pm .19.$

Mean ½-hr. space of dilution $= \frac{20\%}{.91 \pm .19} = (22 \pm 4)\%$ avge. normal body wt. for ht. and age.

* Relative area $= \frac{\text{Area under dilution curve of patient}}{\text{Area under the reference curve (1)}}$ for same time interval.

† Relative weight $= \frac{\text{Wt. of subject}}{\text{Average normal wt. for ht. and age}}$

DISCUSSION

The results of this study indicate that there is no significant sex difference in the mean half-hour extrathyroidal space of radioiodide dilution, and confirm the value of (20 ± 2) per cent of actual body weight previously obtained. Edematous subjects were excluded from this study, since it is clearly to be expected that their radioiodide spaces would vary markedly with the amount of edema. However, for subjects outside the normal weight range and for those subjects who have lost weight during the course of a disease such as hyperthyroidism, the mean space of radioiodide dilu-

TABLE 4

Subjects	Mean half-hour radioiodide space	
	Per cent actual body wt.	Per cent avge. normal body wt. for ht. and age
Overweight	16	21
Underweight	25	18
Hyperthyroid	25	22

tion is better correlated with the average normal weight based on the subject's height and age. Calculation of the radioiodide space in this way implies that the gain or loss of weight occurs in a body compartment which contains only a negligible amount of rapidly equilibrating iodide. Since most of the departures from normal body weight are due to differences in body fat, these results suggest that in body fat the iodide is either low in concentration or slow to equilibrate. It should be noted, however, that the correction factor may overcorrect, though only slightly, the value for the space of radioiodide dilution.

The question of especial clinical interest is whether or not indirect determination of the mean plasma concentration from the body-weight value is likely to introduce a nonthyroidal factor which might reduce the reliability of the thyroidal iodide clearance test as an index of thyroid function. Direct determination of mean plasma concentration for the initial half-hour period following intravenous administration of the radioiodide would require the assay of arterial samples for the first minute or so and multiple venous samples during the rest of the time, because of the rapid change of radioiodide concentration. Such a complex technical procedure would make routine thyroidal clearance determinations impractical. This study indicates that if a value be used for the mean half-hour space of radioiodide dilution which is 20 per cent of the actual weight for subjects in the normal weight range and 20 per cent of the average weight based on height and age for all other subjects (including those with marked weight loss although still in the normal range) it will be so close to the true value for the space of dilution that it is not likely to introduce an error large enough to cause overlapping between euthyroid (upper limit, 50 ml./min.) and hyperthyroid (lower limit, 70 ml./min.) thyroidal iodide clearance values.

SUMMARY

1. For both male and female subjects within the normal weight range, the half-hour space of dilution of radioiodide is (20 ± 2) per cent of the body weight.

2. For all subjects outside the normal weight range, the mean half-hour space of radioiodide distribution is better correlated with the average weight for the subject's height and age.

3. An indirect determination of the mean plasma concentration from the body weight value is not likely to reduce the reliability of the thyroidal iodide clearance test as an index of thyroid function.

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Letters to the Editor

THE REGULAR OCCURRENCE OF 3α-ALLOTETRAHYDROCORTISOL (3α,11β,17α-21-TETRAHYDROXY-ALLOPREGNAN-20-ONE) IN HUMAN URINE*

TO THE EDITOR:

In a paper, "The Determination of Tetrahydrocortisone and Tetrahydrocortisol in Urine of Normal and Schizophrenic Men" (in press in this journal), the isolation of 3α-allo-tetrahydrocortisol (allo-THF) is being reported. Since that time a series of 24-hour and 12-hour urine samples from 10 normal and 5 schizophrenic men between the ages of 20 and 39 have been analyzed for the presence of this steroid. The method described in the aforementioned paper (1) was used, with the exception that some 24-hour collections were continuously extracted in a Hershberg-Wolfe apparatus with methylene chloride (2). In order to separate the allo-THF from tetrahydrocortisone (THE) with which it is associated after paper chromatography in the benzene:formamide system, an additional separation of these two compounds was made by subjecting the eluted combination to further paper chromatography in the chloroform:formamide system (3) for twenty-four hours. The eluted tetrahydrocortisol (THF) was also rechromatographed in this solvent system.

For each of the 5 schizophrenic and each of the 10 normal male subjects the THE area eluted from the benzene:formamide paper chromatogram separated in the chloroform:formamide system into 2 blue-tetrazolium (BTZ) (4) reacting spots—one having a running rate similar to that of THE, and the other having a rate, slower than that of THE. This more polar material, when re-run on the same chromatogram with THF, had a running rate 1.3 times faster than that of THF. From each individual's chromatograms, aliquots were taken of the 3 eluted BTZ-positive areas for determination of sulfuric-acid chromogen spectra (5). THF and the presumed allo-THF had maxima of 310–330 mμ, 410–415 mμ, and 505–510 mμ, whereas THE had maxima of 310–330 mμ and 410–415 mμ.

In addition, a sufficient amount of the tentatively identified allo-THF was present in eluates of paper chromatograms from 2 schizophrenic and 5

* Aided by grants from the Albert and Mary Lasker Foundation and Mrs. Henriette Durham.

normal samples to obtain crystalline material. A readily crystallizable substance was also obtained from both a pool of eluates from the other 3 schizophrenics and from 2 normal samples. Each of the 9 crystalline samples submitted for infrared analysis was identified as 3α,11β,17α,21-tetrahydroxyallopregnan-20-one.

Porter-Silber (PS) determinations (1) were also made on aliquots of the 3 eluted steroids. These values, corrected as previously described for aliquots taken, removal of test strips, and loss due to paper chromatography, are expressed both as ratios of total PS excretion of the 3 steroids and as absolute amounts in milligrams per twenty-four hours.

The mean ratios of excretion of each of the 3 steroids to their total excretion in 24-hour samples extracted continuously with methylene chloride

TABLE 1. MEAN RATIOS OF EXCRETION OF THF, ALLO-THF AND THE PER TWENTY-FOUR HOURS, AS DETERMINED BY THE PORTER-SILBER REACTION

	N	Benzene: Formamide system		Chloroform: Formamide system					
		% of THF*+allo-THF†+THE in THE equivalents		% of THF+allo-THF+THE					
				In THE equivalents			Each steroid in own equivalents		
Methylene chloride extraction		THF	allo-THF+THE	THF	allo-THF	THE	THE*	allo-THF†	THE
Schizophrenics	5	14.4	85.6	15.2	10.8	74.1	15.2	27.1	57.7
Normal subjects	4	18.8	81.5	16.2	11.0	72.8	16.4	27.7	55.9
Ethyl acetate extraction									
Normal subjects	6	25.1	74.9	24.5	8.1	67.4	25.2	21.8	53.0

* THF—Optical density for equivalent concentrations =75% of that for THE (tetrahydrocortisone).
† Allo-THE—Optical density for equivalent concentrations =29% of that for THE (tetrahydrocortisone). (Mean optical density from 3 standard curves =28.6%; S.E. ±0.03).

appear in Table 1. There was no substantial difference between the normal and schizophrenic men in the distribution of these compounds in urine. Since the excretion ratios of THF and THF plus allo-THF were different from those found with ethyl acetate extraction (1), half of a 24-hour urine sample from each of 6 young men was extracted as six 2-hour aliquots with ethyl acetate. The aliquot extracts were combined and the 12-hour equivalent residue submitted to paper chromatography. The resulting mean ratios of excretion of THF (25.1 per cent) and THE plus allo-THF (74.9 per cent) to the total excretion of these 3 metabolites, were similar to those previously obtained (25.4 per cent and 74.6 per cent, respectively). Approximately 22 per cent of the combined excretion of these steroids was accounted for as allo-THF.

TABLE 2. MEAN EXCRETION VALUES (MG./24 HRS.) OF TOTAL PS, THF, ALLO-THF AND THE IN 6 NORMAL MEN

	Values expressed in THE equivalents		Values for each steroid expressed in own equivalents	
	Mean	Range	Mean	Range
Total PS	5.5	3.5–7.1	—	—
THF+Allo-THF+THE	4.5	2.7–6.4	5.7	3.5–8.0
THF	1.0	0.7–1.4	1.3	0.9–1.9
Allo-THF	0.4	0.2–0.6	1.3	0.6–2.1
THE	3.1	1.6–5.3	3.1	1.6–5.3

The absolute output of total PS, THF, allo-THF, and THE are presented in Table 2. The mean values for the 6 men were lower than those previously indicated; however, their range was within the limits previously found. The absolute outputs of allo-THF varied from 0.6 to 2.1 mg., with a mean excretion of 1.3 mg. per twenty-four hours. Based on previous results, in a larger male population the upper limit would probably be at least 2.5 mg., with a mean output of approximately 1.5 mg. per twenty-four hours.

In view of the occurrence of allo-THF in urine, the comparable isomer of tetrahydrocortisone, allopregnanetriol-3α,17α,21-dione-11,20 (allo-THE) was sought. In 12-hour urine extracts only trace amounts of a BTZ-reacting substance was found in the area of paper chromatograms where allo-THE might be expected to occur. In several accumulations of this material, each equivalent to a collection of three to four days, there was no evidence of the presence of allo-THE. Under the circumstances of these experiments, allo-THE, if present, is in amounts considerably less than those of allo-THF.

SUMMARY

A corticosteroid of the allo series, 3α-allotetrahydrocortisol, has been found in measurable amounts in urinary extracts of 5 schizophrenic and 10 normal men, and is presumably a regularly occurring metabolite in men. It constitutes approximately 22 per cent of the absolute output of tetrahydrocortisol, 3α-allotetrahydrocortisol and tetrahydrocortisone combined.

LOUISE P. ROMANOFF, B.A.
JOYCE SEELYE, M.A.
ROSA RODRIGUEZ, M.A.
GREGORY PINCUS, D.SC.

Worcester Foundation for Experimental Biology,
Shrewsbury, Massachusetts

Acknowledgments

The authors wish to express their thanks to Mr. Paul Potvin for determinations of the infrared spectra and to Dr. Harold Levy for allopregnanetetrol-3α, 11β, 17α, 21-one-20.

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THE CONVERSION OF 4-C^{14}-CORTISOL TO C^{14}-17-KETOSTEROIDS

To the Editor:

The concentration of urinary 17-ketosteroids (17-KS) increases during the administration of large doses of 17-hydroxycorticosteroids (17-OHCS: cortisone or cortisol) to human subjects. Dorfman and his associates (1, 2) have characterized the 17-KS present in the urine following the administration of large doses of cortisol or cortisone and their findings indicate that the 11-oxygenated etiocholane derivatives constitute the major part of the 17-KS originating from the 17-OHCS. Even though the foregoing studies pointed strongly to the probability that the 17-KS do, in fact, originate from the 17-OHCS, definite proof of their origin has awaited verification by isotopic studies. In this communication we wish to present evidence for the conversion of 4-C^{14}-cortisol to C^{14}-17-ketosteroids in human subjects.

METHODS

Patients receiving large doses of cortisol or prednisone orally (30–40 mg. every three hours) for at least four days were given 1.6 μc. of 4-C^{14}-cortisol[1] (10 μc. per 2.45 mg.) intravenously. Urine was collected during the following twenty-four to forty-eight hours and the conjugates were hydrolyzed either by adding 15 per cent by volume of concentrated HCl to boiling urine and refluxing for ten minutes or by mild methods previously

[1] 4-C^{14}-cortisol was kindly supplied by the Endocrinology Section of the National Institutes of Health.

described (3). After extraction of the 17-ketosteroids (17-KS) with CH_2Cl_2, the extract was washed with small volumes of alkali and water.

Boiling acid destroys the C_{21} metabolites of cortisol, but mild hydrolysis does not. To separate the C_{19} metabolites of cortisol from the C_{21} metabolites, the neutral extracts were chromatographed on Florisil. For the sake of uniformity, extracts obtained after both acid and mild hydrolysis were processed in this manner. The procedure of Glenn and Nelson (4) was simply scaled up in order to handle extracts of a complete twenty-four or forty-eight hour urine. The extracts dissolved in a small volume of CH_2Cl_2 or $CHCl_3$ were poured into a column containing 50 Gm. of Florisil. After the sample and two rinsings had percolated into the column, the 17-ketosteroids were eluted with 400 ml. of solvent containing 2 per cent of CH_3OH. This fraction was then submitted to gradient elution chromatography (5).

The eluants from the column were analyzed for both Zimmermann chromogenicity and radioactivity. If possible, the material under each peak was identified by infrared spectroscopy and/or paper chromatography. The infrared spectroscopy was performed with the KBr technique (6). Paper chromatography was performed according to the methods of Bush (7): each 17-KS was localized and the radioactivity on the paper-strip determined with an Actigraph (Nuclear-Chicago C-100). For example, all peaks IV and V not only had infrared spectra characteristic of 3α-hydroxyetiocholane-11,17-dione and

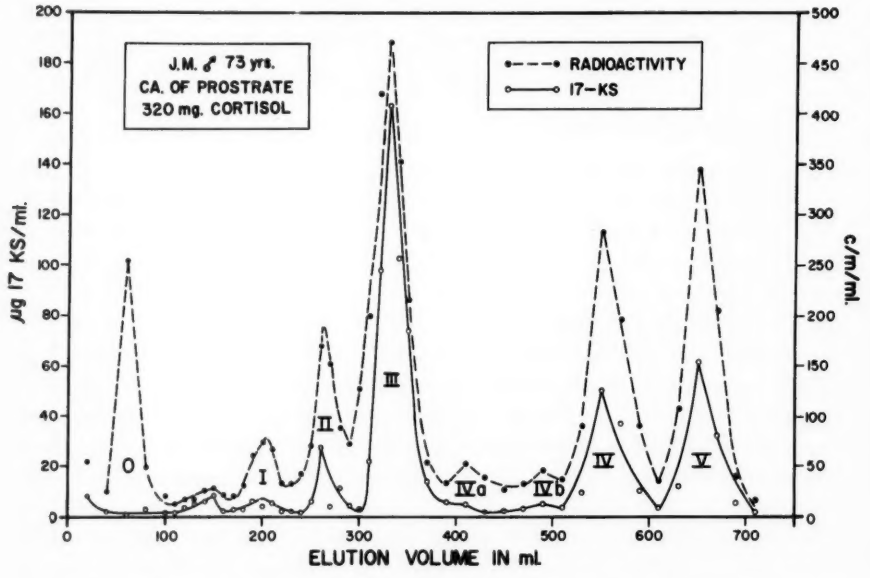

FIG. 1. (Patient J.M., aged 73.) Analysis of urinary 17-KS and radioactivity by gradient elution chromatography. Peak 0 was not identified. Under normal circumstances the peaks represent: I, dehydroisoandrosterone; II, androsterone or 3α-hydroxy-$\Delta^{9(11)}$-androsten-17-one; III, etiocholanolone or 3α-hydroxy-$\Delta^{9(11)}$-etiocholen-17-one; IVa, 11-ketoandrosterone; IVb, 11-β-hydroxyandosterone; IV, 3α-hydroxyetiocholane-11,17-dione; V, 3α,11β-dihydroxyetiocholane-17-one.

TABLE 1. DISTRIBUTION OF RADIOACTIVITY AND 17-KS SHOWN BY ELUTION CHROMATOGRAPHY OF URINARY EXTRACTS OF SUBJECTS RECEIVING C^{14}-CORTISOL INTRAVENOUSLY

	Mild hydrolysis				*Acid hydrolysis*			
Subject's Sex and Age; daily dose of steroid; diagnosis→	F.U. ♂ 66; 240 mg. Δ^1E; Ca. of prostate (orchidect'd.)		C.H. ♂ 62; 240 mg. Δ^1E; chr. lymph. leukemia		J.M. ♂ 73; 320 mg. F; Ca. of prostate		J.H. ♂ 17; 240 mg. F; *normal*	
	c/m $\times 10^{-3}$	17-KS (mg.)	c/m $\times 10^{-3}$	17-KS (mg.)	c/m $\times 10^{-3}$	17-KS (mg.)	c/m $\times 10^{-3}$	17-KS (mg.)
2% Methanol fraction* (% of injected counts)	196 (9.7%)	8.0	60.3 (2%)	8.1	137 (6.7%)	27.4	82.5 (4.2%)	12.2
I†	—	—	—	—	5.7	0.44	—	2.7
II	—	—	—	—	12.6	0.68	14.2	1.7
III	—	—	—	—	36.6	10.00	7.4	0.17
IVa	—	—	—	—	4.7	0.36	11.2	0.66
IVb	—	—	—	—	4.6	0.44	24.2	1.7
IV	54.4	2.3	13.6	1.9	26.0	4.42	6.1	0.24
V	70.4	2.8	18.3	1.5	30.0	4.94	24.2	0.12

* Represents the fraction containing the 17-KS following Florisil chromatography.
† Under normal circumstances represents area of dehydroisoandrosterone.

3α,11β-dihydroxyetiocholan-17-one, but in addition both of the substances had Rf's identical with those of the standards and the radioactivity was located in areas with identical Rf's.

RESULTS AND DISCUSSION

The results in 4 men are shown in Figure 1 and Table 1. It is interesting that the preponderant parts of the radioactivity and 17-KS following mild hydrolysis of the urine were located under peaks IV and V, 3α-hydroxyetiocholane-11,17-dione and 3α,11β-dihydroxyetiocholan-17-one, respectively. On the other hand, following strong acid hydrolysis substantial radioactivity and 17-KS were found under peaks II, III, IVa and IVb. Peaks II and III are thought to represent primarily the C-9:C-11 dehydration products of 11β-hydroxyandrosterone and 3α,11β-dihydroxyetiocholan-17-one respectively, and have been shown to be by-products of the strong acid hydrolysis of the parent compounds in the urine (8). Peaks IVa and IVb were not identified definitely because of lack of standards. It is possible that they represent 11-keto and 11β-hydroxyandrosterone derived from cortisol.

In this study the subjects received oral cortisol or prednisone for long enough periods to produce suppression of adrenocortical function and hence it would seem safe to assume that the adrenal cortex contributed little to the 11-oxygenated 17-KS in the urine of these subjects. In addition, the amount of Zimmermann chromogens present in the urine following

prednisone administration is lower than that following cortisol. We have reported on this observation previously and pointed out that the conversion of 4-C^{14}-cortisol to C^{14}-17-KS was not affected by the high doses of prednisone (9).

In 1 subject (C. H.) substantial radioactivity was present under two peaks (VI and VII) when the gradient elution was carried further than in the other subjects. A comparable concentration of Zimmermann chromogens was not present under these peaks.

CONCLUSION

Human subjects convert a significant amount of intravenously injected 4-C^{14}-cortisol to C^{14}-17-ketosteroids. The majority of these 17-ketosteroids belong to the 11-oxygenated etiocholane group.

AVERY A. SANDBERG, M.D.
EDWARD CHANG, M.S.
W. ROY SLAUNWHITE, JR., PH.D.

Roswell Park Memorial Institute,
Buffalo 3, N. Y.

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THE EXCHANGEABLE CHARACTER OF ACCUMULATED I^{131} IN THE THYROID GLAND OF A GOITROUS CRETIN

To the Editor:

It has been reported that although thyroid hormone cannot be synthethesized by the thyroid gland of a goitrous cretin, the thyroid uptake of I^{131} is usually high (1–6). Stanbury and Hedge (1, 2) demonstrated that the accumulated thyroidal I^{131} can be readily discharged by means of the administration of thiocyanate, indicating that the trapped I^{131} remains in the form of iodide and is diffusible. This suggests that a defect exists in the conversion of iodide into an organically bound form, and that the large goiters are the result of compensatory hyperplasia. The dissociation of the processes of iodide trapping and hormonal synthesis in goitrous cretins supports the concept of the existence of separate mechanisms for these functions in the normal thyroid gland.

We have had the opportunity to study a goitrous cretin in our laboratory. Although Stanbury and his associates have described the behavior of thyroidal I^{131} in this disorder, the number of subjects studied has been small, and we feel that a report of our observations in an additional case will be of interest. Unfortunately, it was not possible to obtain studies on the 3 cretin siblings of our patient. The methods used for determination of the thyroidal I^{131} uptake, the plasma protein-bound I^{131} level, and the serum chemical protein-bound iodine level have been described previously (7, 8).

The following is a summary of the clinical data in our case:

The patient was a 34-year-old white male, one of 13 children, of whom 3 others were cretins. He was recognized as a cretin shortly after birth. However, due to economic factors, thyroid therapy was only sporadic until he reached the age of 5 years. At that time a regular schedule of desiccated thyroid was started, in a dosage of 120 mg. daily.

When first seen by us, his thyroid gland had been enlarged for fifteen years. For the preceding three years it had gradually increased in size and was causing difficulty in swallowing and breathing. He had attended school for three years but could not read or write, and was emotionally very labile and easily upset.

Physical examination revealed a well nourished male, who spoke haltingly and was obviously markedly retarded mentally. The skin was warm and moist, the hair normal, and the tongue normal in size. The blood pressure was 120/76 and the pulse 86. There was a coarse tremor of the hands and unsustained knee and ankle clonus. The thyroid gland was firm and massively enlarged with multiple large confluent nodules, particularly in the right lobe. The appearance of the patient and his goiter are shown in Figure 1. The remainder of the physical examination showed nothing abnormal.

The BMR was −4 per cent, but the patient cooperated poorly during the test. The level of hemoglobin was 17.6 Gm. per cent: of serum cholesterol, 104 mg. per 100 ml.; of calcium, 11.5 mg. per 100 ml.; of phosphorus, 2.8 mg. per 100 ml.; and of alkaline phos-

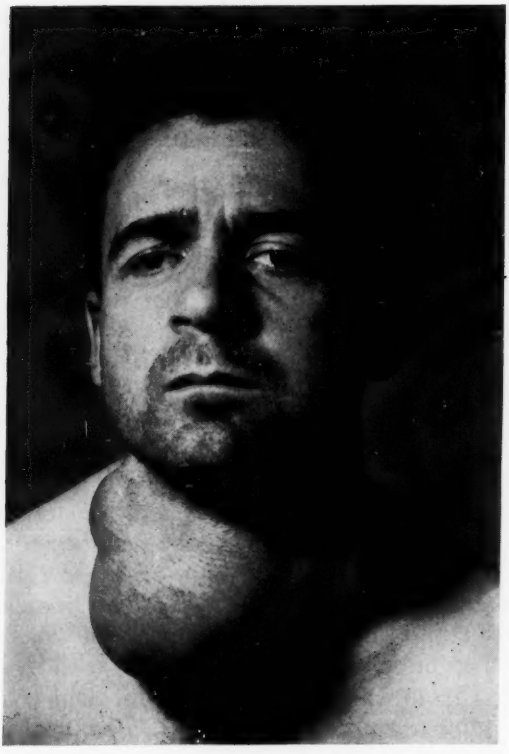

FIG. 1. A 34-year-old goitrous cretin. The goiter (removed by thyroidectomy) weighed 647 grams and manifested adenomatous, cystic and degenerative changes.

phatase, 6.3 King-Armstrong units. An electrocardiogram was normal. A roentgenogram and fluoroscopy of the chest revealed a large goiter mass containing stippled calcifications, in the anterior neck and superior mediastinum. The trachea was compressed and deviated to the left.

At operation, a large lobular and nodular goiter, weighing 647 grams and measuring 10×13×13 cm., was removed. Microscopically, the tissue exhibited extensive necrosis and degeneration, and areas of hemorrhage. The cellular pattern was variable, some areas being adenomatous with regular acini lined by tall cells, and other areas containing large colloid cysts or fetal adenomas with small acini in an acellular matrix.

The patient made an uneventful recovery from the operation and was discharged on a regimen of desiccated thyroid, 120 mg. daily.

The thyroid function studies illustrated in Figure 2 were performed prior to surgery. At twenty-four hours the thyroidal I^{131} uptake was 22 per cent. Following an oral dose[1] of 300 microcuries of I^{131}, the thyroidal I^{131} rapidly increased to 33 per cent at three hours and remained at approximately this

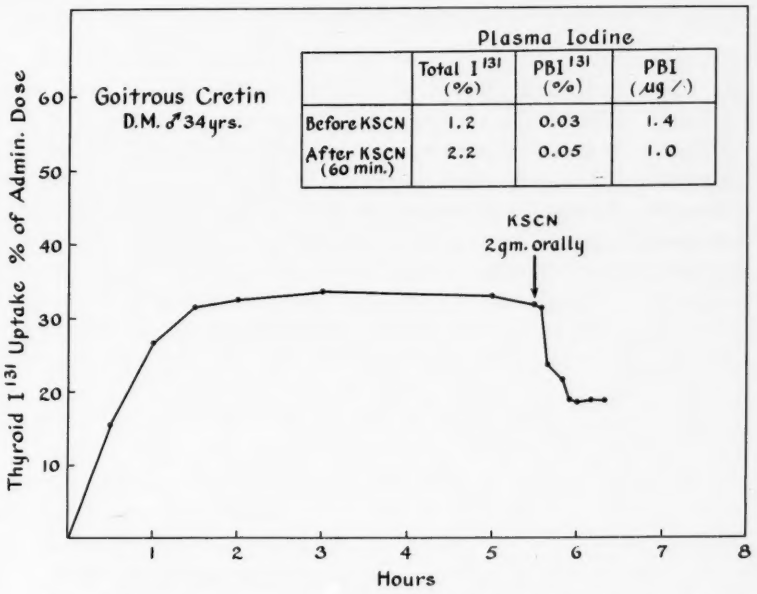

FIG. 2. The effect of thiocyanate administration on the accumulated I^{131} in the thyroid gland of a goitrous cretin. Plasma total I^{131} and protein-bound I^{131} are expressed as a percentage of the tracer dose of I^{131} per liter of plasma.

level over the subsequent two and a half hours. In our laboratory, euthyroid subjects usually have thyroidal I^{131} uptakes less than 20 per cent at three hours. A dose of 2 Gm. of thiocyanate was given orally five and a half hours after the second I^{131} tracer. There was an immediate and rapid decrease in thyroidal I^{131}, and at thirty minutes approximately half of the accumulated I^{131} had been discharged from the gland. No further decrease occurred over the next thirty minutes, at which time observations were discontinued. Sixty minutes after thiocyanate administration the plasma total I^{131} content had almost doubled; however, there was no significant change in the level of plasma protein-bound I^{131}. The serum chemical protein-bound iodine level was 1.4 µg. per 100 ml., and there was no significant change after thiocyanate administration.

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[1] The 24-hour thyroidal uptake of 22 per cent was determined on a separate tracer dose given five days before the 300-mc. dose of I^{131}. The 3-hour uptake and the KCNS studies were carried out following the second dose. A thyroid and blood I^{131} radioactivity background was measured before the second dose was given, and all measurements were corrected for this background.

CONCLUSIONS

Our observations agree with those of Stanbury and Hedge (1, 2), and indicate that the thyroid glands of goitrous cretins have a supernormal avidity for I^{131}. After being trapped, the I^{131} remains predominantly in the form of iodide, is freely diffusible, and can be discharged from the gland by thiocyanate. The same phenomenon is observed in glands blocked by antithyroid drugs, but not in normal glands. The high thyroidal I^{131} uptake at three hours followed by a plateau and a normal value at twenty-four hours suggests that, although a large quantity of I^{131} is trapped, it cannot be incorporated into thyroid hormone. Thus a continual interchange between thyroidal and plasma I^{131} occurs, and the accumulated thyroidal I^{131} does not progressively increase as might be expected from the high three-hour value. These observations are further evidence that separate mechanisms for iodide trapping and hormone synthesis exist in the thyroid gland.

ALVIN L. SCHULTZ, M.D.
EDMUND B. FLINK, M.D.
BYRL J. KENNEDY, M.D.
LESLIE ZIEVE, M.D.

Department of Medicine and
the Radioisotope Service of
the Veterans Administration Hospital and
the University of Minnesota,
Minneapolis, Minnesota

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CONTROL OF DIABETES INSIPIDUS BY THE BUCCAL ADMINISTRATION OF POSTERIOR PITUITARY POWDER (PITRESSIN)

To the Editor:

We have observed 2 children with diabetes insipidus whose mothers were or became allergic to the posterior pituitary powder (Pitressin) administered by insufflation to the children. Since neither child could be depended upon to administer the insufflation of Pitressin powder, an alternative method of control of the diabetes insipidus had to be discovered.

We tried to avoid the use of injections, for obvious reasons. From lactose and the posterior pituitary powder that we had used as snuff, the hospital pharmacist made tablets which we gave to one of these children with instructions to let the tablet dissolve between her teeth and her cheek. Buccal absorption proved effective in this case. When she inserted four of the tablets (approximately 20 mg. each) between the teeth and buccal surfaces before she went to sleep at night, she was able to sleep though the night without wetting the bed or having to get up to void or drink water. She would then receive two insufflations of powder when she went to school. This provided good control until late in the evening, just before retiring. Formerly the maintenance dose had been three insufflations of Pitressin snuff, in doses of approximately 20, 20 and 40 mg. respectively. Because the mother was so appreciative of the results, we felt that in certain cases there might be some merit in this mode of administration of Pitressin.

The Lilly Research Laboratories kindly consented to make some buccal tablets for us. These tablets contain either 100 or 127 mg. of posterior pituitary powder, and are roughly 9 mm. in diameter. They have an unpleasant taste, but this is not evident when they are inserted in the buccal pouch lateral to the bicuspids or molars. They dissolve very slowly, frequently lasting over an hour. Some children were unable to refrain from sucking them; in these instances they dissolved more rapidly and had less effect.

Table 1 shows the effect of the tablets on urinary specific gravity following the ingestion of approximately 1 liter of water as a priming load and a constant intake of 400 ml. per hour thereafter in 2 normal adult men (the investigators).

Table 2 shows the results in 10 children with diabetes insipidus. All the patients had a urinary specific gravity less than 1.004 before receiving posterior pituitary therapy. In the 3 out-patients, control was considered adequate if the mother thought the results were as good as with the previous medication and the children did not require extra bathroom privileges at school and did not have to void more than once during the night after re-

TABLE 1. EFFECT OF BUCCAL POSTERIOR PITUITARY POWDER ON SPECIFIC GRAVITY OF URINE IN HYDRATED NORMAL ADULTS

Time (min.)	Dose of post. pit. powder	Urine		Comment
		ml./min.	sp. gr.	
R.K. *1000 ml. of water as priming load—400 ml./hr. thereafter*				
60		4.0	1.004	
60		1.3	1.012	
60		7.2	1.000	
	254 mg. (2 tablets)			
30		7.5	1.000	
30		3.2	1.004	
30		1.8	1.010	1 tablet dissolved
30		3.0	1.008	$\frac{1}{3}$ tablet removed
60		1.4	1.015	
Z.L. *800 ml. of water as priming load—400 ml./hr. thereafter*				
90		3.1	1.004	
45		3.8	1.002	
	254 mg.			
45		3.4	1.004	
60		0.5	1.020	
60		0.4	1.022	
60		1.1	1.007	
120		0.8	1.006	

tiring. There were 3 failures among these 10 cases. One 7-year-old boy refused to take the tablets. In the other 2 cases of failure, it is possible that the children did not allow the tablets to dissolve slowly enough to allow for sufficient absorption. Results were best in a comatose patient who did not suck the tablets. The 3 ambulatory patients preferred to control the diabetes insipidus with the tablets rather than with snuff or injections of Pitressin tannate in oil. The 2 mothers who were allergic to the posterior pituitary powder when it was insufflated in their children's nostrils had no trouble when the children used the tablets. The tablets did not give rise to dust in the atmosphere and the mothers did not even need to handle them,

TABLE 2. EFFECT OF BUCCAL POSTERIOR PITUITARY POWDER (PITRESSIN) ON SPECIFIC GRAVITY OF URINE IN CHILDREN WITH DIABETES INSIPIDUS

Patient	Age (yrs.)	Etiology of diabetes insipidus	Buccal administration			Other routes
			Single dose (mg.)	Sp. gr. of urine (highest)	Dosage req'd for 24-hr. control	Dosage req'd for 24-hr. control
Vo	11 1/2	Postoperative craniopharyngioma	100	1.020	100, 100, 200 mg.	1 ml. Pitressin tannate in oil
Wi	11	Postoperative craniopharyngioma	127	1.014	127 mg. t.i.d.	0.5 ml. Pitressin tannate in oil
Ty	7	Postoperative craniopharyngioma	127		Sucked one tablet and refused to take more	20, 20, 40 mg. snuff*
La	12	Postoperative craniopharyngioma	127	1.008	Repeated dosage had less effect	20, 20, 40 mg. snuff
Ja	8	Encephalitis	127	1.024	127 mg. b.i.d.	1 ml. Pitressin tannate in oil
Pe	13	Postoperative craniopharyngioma	100	1.020	100 mg. q. 6 hrs.	80 mg. snuff t.i.d.
Tr	15	Postoperative craniopharyngioma	508	1.010		40, 40, 80 mg. snuff
Li	9	Eosinophilic granuloma		Adequate control, as out-patient	63, 63, 127 mg	20 mg. snuff b.i.d.
To	8	Eosinophilic granuloma		Adequate control, as out-patient	127 mg. b.i.d.	60, 40, 40 mg. snuff
Jo	11	Eosinophilic granuloma		Adequate control, as out-patient	127 mg. t.i.d.	20, 20, 40 mg. snuff

* Posterior pituitary powder by nasal insufflation.

as the children were quite capable of taking the posterior pituitary powder in this form and did so willingly.

SUMMARY

Posterior pituitary powder in dosages of 80 to 500 mg. daily has been administered in the form of buccal tablets to 10 children with diabetes insipidus of pituitary origin. In 7 of them, there has been adequate control of the disease. The 3 failures were presumably due to lack of adequate absorption by the buccal route, in patients who did not cooperate. The children in whom the disease was controlled while at home by use of these tablets preferred it to other common forms of treatment. The buccal tablets were originally designed for, and proved to have the greatest value in those

patients whose mothers suffered from asthmatic attacks precipitated by the use of posterior pituitary powder insufflations in their homes.

ROBERT KLEIN, M.D.
ZVI LARON, M.D.

Department of Pediatrics,
University of Pittsburgh School of Medicine
and the Children's Hospital of Pittsburgh,
Pittsburgh, Pennsylvania

Addendum

It has come to our attention that Cannon (1) has reported being able to control diabetes insipidus by the sublingual administration of posterior pituitary "lozenges" in 4 patients.

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OXYTOCIN AND LACTATION

TO THE EDITOR:

Suckling stimulates ejection of milk—the action occurring by way of a neurohumoral reflex. Although during lactation there is evidence of increased oxytocic activity in the circulation (1), oxytocin has not been identified as such in the blood.

A method for the extraction of oxytocin from rat's blood has been reported by Bisset and Walker (2). Using a modification of this method, we have extracted oxytocin from blood collected from healthy nonpregnant women, and from women towards the end of normal pregnancy and during labor. In this paper, we record the results of the determination of the oxytocic activity of blood taken from women in the first week post partum, before and during suckling, and we postulate the presence of a second oxytocic hormone.

MATERIALS AND METHODS

Samples of blood were collected from the antecubital veins of women who had born a child from four to seven days previously. Ten minutes after collection the infants were suckled. Five minutes after suckling commenced (and during suckling) a further sample of blood was taken from each mother. The bloods were added immediately to acid-alcohol and extracted according to the authors' modification (3) of the method of Bisset and Walker (2). The concentrated extracts were assayed on the isolated uterus of virgin Wistar-strain rats in estrus (4, 5). The possibility of 5-hydroxytryptamine in the extracts

acting as an oxytocic substance (6) was eliminated by dialysis and/or by adding Dibenamine hydrochloride in a concentration of 5 μg./ml. to the organ bath. The potassium concentration of several extracts was estimated directly by a flame photometer, and the amounts of this ion contained in the largest doses of extract used were compared with the amount in the uterine threshold dose of potassium. The highest value for potassium was 0.015 mEq. from an undialyzed sample, and the lowest threshold dose was 0.1 mEq. Thus potassium was not an interfering oxytocic substance in these experiments. A control oxytocin dialysis was run when the extracts were dialyzed, and the average loss of oxytocin through the membrane was 50 per cent. A specimen of each extract was treated with sodium thioglycollate (0.01 M at pH 7.5 for thirty minutes) in order to inactivate oxytocin (7, 8). Samples of some of the extracts were incubated with pregnancy plasma which contains an enzyme which destroys oxytocin (alpha-hypophamine; Pitocin) (9).

RESULTS AND DISCUSSION

In Table 1 it may be seen that 10 extracts were tested for both total oxytocic activity and oxytocin and that, during suckling, the total oxytocic activity increased in 7 and decreased in 3. In the latter group, there was no oxytocin in 2 samples; in the third, 21 per cent of the activity was due to oxytocin. Altogether, 14 samples were treated with thioglycollate. In 12 of these, most of the oxytocic activity persisted. This activity, however, was largely destroyed by an enzyme in pregnancy plasma which is known to destroy oxytocin (9). In Subjects 3, 4, 7, 9 and 10 before suckling, both oxytocin and a second oxytocic substance were present; in Subjects 4, 7 and 10 during suckling, only the second oxytocin substance was present. Since the concentration of this unidentified substance increased during suckling, there is a strong suggestion that it originated in the hypothalamic-posterior pituitary system. On the other hand, it might be simply a nonspecific substance. Its pharmacologic properties are now being investigated. To eliminate the possibility that some substances in the extracts might protect oxytocin from inactivation by thioglycollate, Pitocin was added to some of the extracts and the oxytocin was found to be completely inactivated by sodium thioglycollate.

This oxytocic substance was not dialyzable through the cellophane membranes used (which were partly permeable to oxytocin). It withstood boiling in neutral solutions for two minutes and, like oxytocin, lost activity on storage at 4°C. in dilute solution.

SUMMARY

After the removal of potassium ion and 5-hydroxytryptamine, 2 oxytocic substances have been extracted from the blood of lactating women; one of these is inactivated by sodium thioglycollate and hence is assumed to be oxytocin; the other is stable in thioglycollate but, like oxytocin, is inactivated by an enzyme present in pregnancy plasma. This unidentified oxytocic substance is probably also formed in the hypothalamic-pars-nervosa

TABLE 1. OXYTOCIC ACTIVITY OF ACID-ALCOHOL EXTRACTS OF BLOOD BEFORE AND DURING SUCKLING

Subject			Total oxytocic activity		Oxytocin*	Per cent oxytocin / Total activity	Per cent 2nd oxytocic substance / Total activity
No.	Before or during suckling		Before treatment with thioglycollate	After treatment with thioglycollate			
			Extract not dialyzed (*m.u./ml. blood*)				
1	Before		0.74				
		During	0.92				
2	Before		1.30				
		During	6.50	>5.70	<0.80	<12.3	>87.7
3	Before			>1.90	<2.50	<56.8	>43.2
			4.40	<3.80	>0.60	>13.6	<86.4
		During	7.60	5.60	2.00	26.3	73.7
4	Before		0.78	<0.58	>0.20	>25.6	<74.4
		During	2.20	2.20	0	0	100.0
5	Before		0.52				
		During	1.10	0.90	0.20	18.2	81.8
			Extract dialyzed (*m.u./ml. blood*)				
6	Before		<0.96				
		During	1.75	>1.10	<0.65	<37.10	>62.90
7	Before		2.85	<1.75	>1.10	>38.6	<61.40
		During	0.90	1.00	0	0	100.0
8	Before		<1.10				
		During	9.40	>8.00	<1.40	<14.9	>85.10
9	Before		3.00	>2.20	<0.80	<26.6	>73.4
		During	0.51	>0.40	<0.11	<21.5	>78.5
10	Before		0.60	<0.40	>0.20	>33.3	<66.7
		During	0.44	0.48	0	0	100.0

* The oxytocin figures for dialyzed extracts are uncorrected for loss through the dialysis membrane, where the mean loss of oxytocin was 50 per cent.

region, since it is found in greater amounts than oxytocin in blood samples taken during suckling.

R. W. HAWKER, M.D.
P. A. ROBERTSON, PH.D.*

Department of Physiology,
University of Queensland,
Brisbane, Australia

Acknowledgment

We wish to thank Professor W. V. Macfarlane for his interest in this work; Miss Vivienne Roberts for her technical assistance; and the National Health and Medical Research Council (Australia) for a grant.

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THE PYROGENIC EFFECT OF ETIOCHOLANOLONE

TO THE EDITOR:

The steroid hormone metabolite etiocholanolone (3α-hydroxyetiocholane-17-one) injected intramuscularly in man consistently produces an acute pyrogenic and inflammatory reaction. This is the first reported instance of a reaction of this type produced by a substance of known chemi-

* Present address: Department of Pharmacology, University of Sydney, N.S.W.

cal structure and of physiologic origin, arising *in vivo* from the biochemical transformation of adrenal and gonadal hormones.

METHOD

Etiocholanolone, prepared by partial synthesis, was purified by column chromatography and repeated fractional crystallization until the physical constants were the same as those of the best samples described. For large doses (100 mg. or more) the steroid was dissolved in sterile, pyrogen-free sesame oil containing 8 per cent benzyl alcohol which had been carefully redistilled in pyrogen-free glassware just prior to use; smaller doses were usually dissolved in sesame oil alone. When the pure steroid alone or dissolved in sesame oil was tested in the standardized rabbit pyrogen assay, 50 mg. produced an average rise in temperature of 0.2° C.—a reaction only slightly greater than that of pyrogen-free distilled water.[1] Both the sesame oil and the benzyl alcohol were tested separately and together in the same human subjects who received the steroid and were found to be inert as pyrogenic or inflammatory agents. The solutions of steroid were stored in sealed, single-dose, glass ampules which had been thoroughly cleaned, washed with pyrogen-free water and heated before introduction of the test solution; the solutions were sterilized by repeated heating at 130° C. The syringes and needles were heated at 300° C. for four hours to destroy possible pyrogen contaminants. As controls for etiocholanolone, as well as to study the structural specificity of this compound in the production of fever, solutions of the three closely related epimers, androsterone, isoandrosterone and 3β-hydroxyetiocholane-17-one, were prepared in sesame oil-benzyl alcohol vehicle with the same rigid precautions.

RESULTS

Volunteers, who were informed of the anticipated reaction, were given doses of etiocholanolone ranging from 5 to 200 mg., either in a single intramuscular injection in 1 ml. of vehicle or in two injections in 0.5 ml. of vehicle. The reactions of all were essentially similar and roughly proportional to the amount of steroid administered. In a typical experiment in which the dose was 100 mg., there were severe shaking chills within six hours, accompanied by marked local swelling, heat and tenderness at the site of injection. During the next five hours, these manifestations continued with weakness, muscular aches and pain, anorexia, sweating, severe headache and a fever of 103°F. Approximately eighteen hours later the systemic reaction had disappeared, and the local soreness about the site of injection disappeared during the following twelve hours. There was a diuresis accompanied by an increased creatinine excretion during the day etiocholanolone was administered and this continued during the following day. An abrupt leukocytosis with a shift to the left developed and subsided in the same fashion as the fever. Although not studied in sufficient detail, a leukopenic

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[1] We are grateful to Dr. P. J. Daughenbaugh, Manager of Pharmaceutical Research, Merck and Company, for his kindness in performing this assay.

phase did not seem to precede the period of leukocytosis. In several subjects an acute thrombocytosis was observed. The eosinophil count was variable, but in 2 subjects who received 20 mg. of etiocholanolone daily for a period of two weeks, a significant eosinophilia was noted.

Two men who had previously been given injections of etiocholanolone received intramuscular control injections of 100 mg. of androsterone (3α-hydroxyandrostane-17-one) and, despite marked apprehension after their previous experience, showed no signs of any reaction. One of these subjects, as well as another normal man, received 100 mg. of 3β-hydroxyetiocholane-17-one without ill effect and with no evidence of a rise in temperature. A total of 2 grams of isoandrosterone was given in daily intramuscular injections of 200 mg. to another man without any untoward effect. Of many other steroids studied, only 3α-hydroxypregnane-20-one produced a pyrogenic reaction similar to that observed with etiocholanolone.

The continued daily administration of etiocholanolone in small doses (approximately 10 mg. per day) produced a continuing fever, which subsided shortly after the injections were discontinued. Tolerance to the intramuscular injection of this material was not observed. Pretreatment with large doses (300 mg. per day) of cortisone for five days diminished significantly the febrile reaction to 25 mg. of etiocholanolone. The same dose of cortisone given on the day of injection alone, however, failed to influence the hyperpyrexia. Aspirin, given simultaneously with the etiocholanolone injections, significantly inhibited the febrile response and the local tenderness. Injection of etiocholanolone in patients with hypertension, rheumatoid arthritis, rheumatic fever, or the nephrotic syndrome produced no apparent effect on the disease and the reactions observed were the same as those seen in normal subjects.

It is evident there is a high degree of chemical specificity associated with the pyrogenic action of etiocholanolone. Further studies designed to explore the mechanisms and significance of these observations are in progress in these laboratories.

ATTALLAH KAPPAS, M.D.
LEON HELLMAN, M.D.
DAVID K. FUKUSHIMA, PH.D.
T. F. GALLAGHER, PH.D.

Divisions of Biophysics,
Steroid Metabolism and Biochemistry,
Sloan-Kettering Institute for Cancer Research,
Sloan-Kettering Division,
Cornell University Medical College,
New York, N. Y.

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SEX CHROMATIN AND INTERSEX

To the Editor:

Grumbach, van Wyk and Wilkins (1) were the first to appreciate the significance of the finding of male nuclear sex in cases of "ovarian agenesis," pointing to the link between this condition and male pseudohermaphrodism. Their attractive theory assumes that the female form is neuter or basic and that some male sex hormone is required to allow male physical differentiation to ensue. Thus, genetic males whose gonads fail to develop acquire the female form; if damage to the differentiating testes occurs slightly later in intra-uterine life, male pseudohermaphrodites result. This theory was supported by the report of a case showing dysgenetic gonads with pseudohermaphroditic external genitalia (1).

At this stage the problem of intersex appeared to be approaching a satisfactory explanation. Male pseudohermaphrodites were the result of gonadal failure in genetic males, with consequent partial feminization. True hermaphrodism was due to a genetic aberration which allowed both male and female gonads to develop in the same individual. The chromosomal pattern might be XXY or a single X; or possibly all sex chromatin might be lacking (2). Female pseudohermaphrodism was also explained in most cases. The work of Wilkins (3, 4), Bongiovanni and Eberlein (5, 6), and Jailer *et al.* (7, 8) not only proved adrenocortical dysfunction, but actually showed the exact nature of the hormonal defect which led to partial masculinization of these female infants. A small group of cases remained, however, in which hyperadrenocorticism could not be demonstrated. This group was explained by a phase of temporary intra-uterine adrenocortical overaction (3)—a supposition not easily refuted.

Closer examination of these theories reveals certain discrepancies:

1. In at least 1 instance of female pseudohermaphrodism a male skin pattern was found (2)—in a patient in whom congenital adrenocortical overfunction had not been demonstrated. This unexpected finding requires explanation.
2. Male pseudohermaphrodites commonly undergo normal male puberty. In the theory of Grumbach *et al.* (1) this form of hermaphrodism results from gonadal failure during embryonic life, allowing sub-

sequent partial feminization. If this were true, male pubertal changes, which depend upon normal gonadal endocrine function, would not be expected.

3. According to the foregoing theory, male pseudohermaphrodism represents a sort of late form of gonadal dysgenesis and should, also, be associated with the high urinary excretion of gonadotropin which accompanies any primary gonadal failure. Studies made on these patients have seldom shown this phenomenon.
4. Some cases have been reported in which thorough search has failed to reveal even the primitive streaks of tissue found in cases of gonadal dysgenesis (9). Such completely agonadal individuals may have female or male external appearance. According to the theory mentioned, one would not expect male differentiation to occur without the masculinizing influence of testes.
5. The most recent criticism of the theory lies in the demonstration that young men with primary hypogonadism (*e.g.*, Klinefelter's syndrome) may have female type nuclear patterns (10–12).

These considerations seem at variance with either the validity of the "sexing" method or the theories of sexual differentiation. Normal males and females have not yet been shown to differ from their expected nuclear patterns. That such a divergence is seen only in people with abnormal sexual development suggests that the fault lies in our theories of sex differentiation rather than in the "sexing" methods.

It has been postulated that a chemical inductor or evocator exerts a very early influence on the primitive genital ridge, stimulating its development into either testis or ovary (13). It appears possible to incorporate such an "evocator" into a theory which would explain some of the difficulties discussed. It is necessary to assume the existence of two evocators —one accounting for the development of ovaries, and the other for the development of testes. One or other of these evocators normally predominates to produce a definitive gonad, the function of which is then the differentiation of the body into male or female form. It is believed that the sex of an infant is predetermined genetically, before any evidence of genital distinction appears. [This theory is supported by studies of cat embryos, which show the nuclear sex difference before the gonads have differentiated (14).]

Normally, in a genetic male, the evocator induces formation of testes. These elaborate a further hormone which induces normal masculinization. What would happen to a genetic male who was influenced unduly by the wrong, female evocator? In such an individual ovaries would develop and, in accordance with the theories of Jost (15) and others, a neuter, female

form would ensue. If this were so, one might expect an occasional "normal" female to show a male type of nuclear pattern. Such an occurrence has not been reported. However, this theory might account for one hitherto unsatisfactorily explained group—female pseudohermaphrodites without demonstrable adrenocortical overactivity. Barr has demonstrated the male sex chromatin pattern in such an individual, despite the presence of ovaries (2). The evocator theory might account for this anomaly.

Genetic females normally are influenced by the female evocator; ovaries develop, and a normal female genital apparatus. Let us consider the influence of a male evocator on a female embryo. The gonads would become

TABLE 1

Theory	GENETIC MALE	GENETIC FEMALE
Male evocator in ascendancy	Male gonads; male external genitalia; male "chromatin" (normal males)	Male gonads (? abnormal); male external genitalia; female "chromatin" (*e.g.*, Klinefelter's syndrome)
Female evocator in ascendancy	Female gonads; female external genitalia; male chromatin. (Female pseudohermaphrodites with male "chromatin" and no evidence of hyperadrenocorticism; "normal" females with male "chromatin" possible ?)	Female gonads; female external genitalia; female "chromatin" (normal females)
Failure of evocator system	Agenetic gonads;* female external genitalia; male "chromatin"	Agenetic gonads;* female external genitalia; female "chromatin"
Failure of developing gonad	Dysgenetic gonads; male pseudohermaphrodites, etc.; male "chromatin"	Dysgenetic gonads; female external genitalia; female "chromatin"
Excess intrauterine androgen	Infant Hercules	Common forms of female pseudohermaphrodites with female "chromatin" and evidence of hyperadrenocorticism
Excess intrauterine estrogen	Estrogen-producing testes; female external genitalia with male gonads and male "chromatin"	

* By "agenetic gonads" we mean gonads which have not developed beyond the primitive ridge. This is in contrast with "dysgenetic gonads" in which some development has occurred.

testes; these might elaborate male hormone of the type necessary for male differentiation. Such an individual would have male external genitalia with a female type nuclear pattern. Although this has not been demonstrated in normal males, it has been shown in some eunuchoid males who have no evidence of intersexual anatomic features.

Finally, if the genital ridge remains uninfluenced (*i.e.*, if the evocator system fails completely), gonads fail to differentiate, and persist in the primitive state as seen in gonadal agenesis. If this occurs in a genetic female, one finds gonadal agenesis in a female with female nuclear structure. If it occurs in a genetic male, one finds gonadal agenesis in an apparent female with male nuclear structure. Further, if the gonad in a genetic male fails and its influence ceases at a slightly later stage of embryonic development, one finds partial differentiation of the genital tract and, perhaps, varieties of male pseudohermaphrodism.

The foregoing considerations are represented in Table 1. Included in this table are the clinical pictures of other intra-uterine hormonal imbalances, *i.e.*, excessive intra-uterine androgen or estrogen production.

We realize that the evocator theory has little to support it on factual grounds, and that it leaves several questions unanswered. We still cannot explain why completely agonadal individuals may have male external genitalia. Nevertheless, we present this theory in the hope that it will help to elucidate a problem which has become most complex.

R. Hoffenberg, M.D.
W. P. U. Jackson, M.D.

Endocrine Clinic,
Department of Medicine,
Groote Schuur Hospital
and University of Cape Town,
Cape Town, South Africa

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The Endocrine Society

THE 1957 ANNUAL MEETING

The Thirty-ninth Annual Meeting of The Endocrine Society will be held in the Hotel New Yorker, New York City, Thursday, Friday and Saturday, *May 30 and 31, and June 1, 1957.*

A joint session will be held with The American Goiter Association on Thursday morning, May 30, and a joint session with The American Diabetes Association on Saturday morning, June 1. Both of these joint sessions will be held in the meeting room of The Endocrine Society in the Hotel New Yorker.

The Committee on Local Arrangements is Dr. Rulon W. Rawson as Chairman with Drs. Earl T. Engle, Joseph W. Jailer, Warren O. Nelson, and Martin Sonenberg as members of the Committee.

All Scientific Sessions will be held in the Hotel New Yorker. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, May 31, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

All members are urged to make hotel reservations immediately inasmuch as the hotels expect to be filled to capacity. Make reservations directly with the New Yorker, advising time of arrival and departure date. Make your reservations now and avoid disappointment.

THE 1957 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations

presented by members of the Society and is limited to citizens of the United States and Canada. Dr. Carl R. Moore was the recipient in 1955 and Dr. Frederick L. Hisaw in 1956.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

The American Goiter Association

THE 1957 ANNUAL MEETING

The next Annual Meeting of The American Goiter Association will be held in the Statler Hotel, New York City, on *May 28, 29 and 30, 1957.*

POST-GRADUATE COURSES IN PEDIATRICS—HARVARD MEDICAL SCHOOL

1. *Pediatrics*—June 3 to 14 inclusive, 1957; daily, 9:15 A.M. to 4:30 P.M., at the Massachusetts General Hospital, Boston.

This course provides a general review, with emphasis on recent advances and therapy. The first seven days are devoted to pediatric medical, surgical, orthopedic, cardiac, allergic, dermatologic, psychiatric, eye, ear, and x-ray problems. During the last four days there will be concentration on disorders of growth and development, common endocrine problems, the metabolic aspects of infant feeding, parenteral fluid therapy, and the management of nephritic and diabetic patients.

2. *Pediatric Metabolism and Endocrinology*—June 11 to 14 inclusive, 1957; daily, 9:15 A.M. to 4:30 P.M., at the Massachusetts General Hospital, Boston.

This course is the same as the last four days of the course in Pediatrics, but may be taken separately.

Those interested should write: *Courses for Graduates, Harvard Medical School, 25 Shattuck Street, Boston, Massachusetts.*

LAURENTIAN HORMONE CONFERENCE

The 1957 Laurentian Hormone Conference of the AAAS will be held at Mont Tremblant Lodge, Mont Tremblant, Quebec, during the period September 1–6, and investigators interested in attending this conference should make application to the Committee on Arrangements of The Laurentian Hormone Conference, 222 Maple Avenue, Shrewsbury, Massachusetts, at an early date and in any event no later than May 10, 1957. A conference rate of $12.00 per person per day is extended to all invited participants. Attendance is limited to invitations issued by the Committee on Arrangements. The following program has been arranged:

PROGRAM FOR 1957

I. HORMONE STRUCTURE AND FUNCTION

The chemistry and biological activities of 16-hydroxylated steroids.
Seymour Bernstein, Lederle Laboratories.
Sunday evening, September 1

Structure and hormonal activity of some new steroids.
Victor Drill and Byron Riegel, G. D. Searle & Co.
Monday morning, September 2

Relation of molecular structure of phenanthrene derivatives to growth.
Charles Huggins and Elwood V. Jensen, University of Chicago.
Monday morning, September 2

Studies on the mechanism of action of estrogen.
Gerald C. Mueller, University of Wisconsin.
Monday evening, September 2

II. Hormones in growth and development

Hormones and protein metabolism.
J. A. Leathem, Rutgers University.
Tuesday morning, September 3

Effects of hormones on growth in fattening and meat production potential of livestock (of domestic animals).
F. X. Gassner, E. C. Reifenstein, Jr., John W. Algeo and W. E. Mattox, Colorado A. & M.
Tuesday morning, September 3

The hormonal control of mammary growth and lactation.
William Lyons, University of Southern California.
Tuesday evening, September 3

III. Aspects of reproduction

Cytological tests of chromosomal sex in relation to sexual abnormalities in man.
M. L. Barr and Melvin Grumbach, University of Western Ontario and Columbia University.
Wednesday morning, September 4

Clinical aspects of sexual abnormalities in man.
Robert Greenblatt, University of Georgia.
Wednesday morning, September 4

IV. Hormones and metabolism

Effects of hormones on human serum lipoproteins.
Howard Eder, Albert Einstein College of Medicine.
Thursday morning, September 5

Effect of hormones on connective tissue metabolism.
Albert Dorfman and Sara Schiller, University of Chicago.
Thursday morning, September 5

Hormonal influences on connective tissue changes in aging.
Harry Sobel and Jessie Marmorston, Cedars of Lebanon Hospital.
Thursday evening, September 5

V. Neurohumors

Distribution and metabolism of adrenergic neurohumors in axones and tissues.
U. S. von Euler, Stockholm, Sweden.
Friday morning, September 6

The excretion of epinephrine and norepinephrine under stress.
Fred Elmadjian, Justin M. Hope, and Edwin T. Lamson, Worcester Foundation for Experimental Biology.
Friday morning, September 6

BOOK REGISTER

Analytical Pathology. Edited by ROBERT C. MELLORS, M.D., PH.D., Associate, Pathology Division, Sloan Kettering Institute for Cancer Research, and Assistant Professor of Pathology, Cornell University Medical College, Sloan-Kettering Division, New York, N. Y., with a Foreword by EUGENE L. OPIE, M.D., SC.D. There are chapters on Cancer, The Cardiovascular System, The Kidney, The Liver, The Endocrine System, The Hematopoietic System, and Hypersensitivity (Connective Tissue), covering detailed analyses of these fields by 9 prominent research workers. Fundamental aspects of the etiology and pathogenesis of disease processes are presented, emphasizing basic aspects and related practical aspects. These reviews stress the contributions that biology, chemistry and physics have made to a better understanding of the medical problems discussed; 477 pages; illustrated; 1957. McGraw-Hill Book Company, Inc., New York, N. Y. Price $12.00.

Ciba Foundation Symposia. Medical Book Department, Little, Brown and Company, Boston, Mass., and J. & A. Churchill, Ltd., London, England.

Bone Structure and Metabolism. Edited by G. E. W. WOLSTENHOLME and C. M. O'CONNOR. Thirty participants present discussions of bone structure, bone salts, histologic remodeling of adult bone, fibrogenesis, mucopolysaccharides, radioautographic studies of matrix formation, metabolism of bone citrate, magnesium and vitamin D, nutrition of bone, repair of fractures, parathyroid function, vascularity, and experimental bone disease. The book covers the subject from anatomy and histology, through biochemistry and physiology, to clinical medicine; 299 pages; illustrated; 1956. Price $8.00.

Paper Electrophoresis. Edited by G. E. W. WOLSTENHOLME and E. C. P. MILLAR. Twenty-two leading experts in the field for the first time present their work together in a volume which covers such topics as general methods of paper electrophoresis and their use in medicine and biochemistry, evaluation of the albumin-globulin ratio of plasma, analysis of human hemoglobins, physicochemical aspects and their relationship to the design of apparatus, and the future of the technic in its application to research and routine analysis; 224 pages; illustrated; 1956. Price $6.75.

Klinische Physiologie und Pathologie. Von PROF. DR. FERDINAND HOFF, Direktor der I. Medizinischen Universitätsklinik, Frankfurt a.M. This 5th edition has been completely re-written. Included in its 15 chapters are presentations of the blood and blood-forming organs, glands of internal secretion and metabolism. The large portion of the book which is concerned with endocrine and metabolic problems makes it particularly valuable for endocrinologists; 1120 pages; 255 illustrations; 1957. Georg Thieme Verlag, Stuttgart. In U.S.A. and Canada—Intercontinental Medical Book Corporation, New York 16, N. Y. Price DM 79.50. $18.95.